CALCITONIN GENE-RELATED PEPTIDE AND ITS BINDING SITES IN THE HUMAN CENTRAL NERVOUS SYSTEM AND PITUITARY

Binding sites for synthetic human ^{125}I-labeled calcitonin gene-related peptide (^{125}I-CGRP) have been demonstrated in membranes of the human nervous system. Binding was high in the cerebellar cortex (1.35 ± 0.27 fmol/mg of tissue; mean ± SEM), spinal cord (1.06 ± 0.27 to 1.27 ± 0.23 fmol/mg), and nucleus dentatus (1.02 ± 0.15 fmol/mg), intermediate in the inferior colliculus (0.80 ± 0.14 fmol/mg) and substantia nigra (0.75 ± 0.14 fmol/mg), low in the neocortex, globus pallidus, nucleus caudatus, hippocampus, amygdala, superior colliculus, thalamus, and hypothalamus (0.15-0.32 fmol/mg), and negligible in spinal and sympathetic ganglia and pituitary ($\leq$ 0.04 fmol/mg). Autoradiography showed distinct ^{125}I-GRP binding over the molecular and Purkinje cell layers of the cerebellar cortex and over the substantia gelatinosa posterior of the spinal cord. The highest levels of CGRP-like components were recognized in the dorsal part of the spinal cord and the pituitary gland. In the ventral part of the spinal cord as well as in the pituitary and thyroid glands, CGRP values were higher when measured by radioreceptor assay as compared to RIA, indicating that at least two CGRP-like components are present. The predominant CGRP-like peak on HPLC had the retention time of synthetic human CGRP. Immunohistochemistry revealed the presence of a dense plexus of CGRP immunoreactive nerve fibers in the dorsal horn of the spinal cord.

Lundberg, J. M. et al.

Proceedings of the National Academy of Sciences of the United States of America 82:248-252, 1985.

Other support: Swiss National Science Foundation; the Canton of Zurich, Switzerland; Ciba-Geigy Company; Swedish Medical Research Council.

From the Research Laboratory for Calcium Metabolism, Departments of Orthopedic Surgery and Medicine, the Institute of Pathology, University of Zurich, Switzerland; Departments of Histology and Pharmacology, Karolinska Institute, Stockholm, Sweden; and the Department of Anatomy, University of Oxford, Oxford, England.

LEUKOTRIENES C_4, D_4 AND E_4 CAUSE WIDESPREAD AND EXTENSIVE PLASMA EXTRAVASATION IN THE GUINEA PIG

1. Intravenous injection of leukotriene C_4 (1 nmol/kg) caused substantial plasma exudation in anesthetized guinea pigs, as evidenced by marked hemoconcentration (15% ... 5 min) and significant extravasation of Evans blue.

2. Fluorometric quantitation of Evans blue content in 38 selected tissues documented that leukotriene C_4 caused significant plasma extravasation throughout the body, except for the brain, stomach, duodenum, colon, and gonads.

3. In particular, the respiratory and the uro-genital tracts, but also the conjunctiva, the esophagus, the bile ducts and the umbilical ligaments, were very sensitive to the edema-promoting effect of leukotriene C_4.

4. Intravenous injection of leukotriene D_4 (1 nmol/kg) or E_4 (5 nmol/kg) evoked plasma extravasation with a distribution and magnitude that was similar to that induced by leukotriene C_4.

5. It is concluded that the three major constituents of slow reacting substance of anaphylaxis (SRS-A), leukotrienes C_4, D_4 and E_4 cause a generalized and extensive plasma exudation that is consistent with the proposal that these leukotrienes are important mediators of inflammation.

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

Hua, X.-Y., Dahlen, S. E., *Lundberg, J. M.*, Hammarstrom, S., and Hedqvist, P.

Archives of Pharmacology 330:136-141, 1985.

Other support: Swedish Medical Research Council; Swedish Society Against Heart and Chest Diseases; Astra Foundation; Harald and Greta Jeanssons Foundation; Swedish Tobacco Company, Petrus and Agusta Hedlunds Foundation; Karolinska Institute; and National Institute of Environmental Medicine.

From the Departments of Pharmacology, Physiology and Physiological Chemistry, Karolinska Institute; and the National Institute of Environmental Medicine, Stockholm, Sweden.

CAPSAICIN TREATMENT DECREASES TISSUE LEVELS OF NEUROKININ A-LIKE IMMUNOREACTIVITY IN THE GUINEA PIG

The tachykinins are a family of naturally occurring bioactive peptides which share a similar C-terminal sequence of amino acid residues and have similar biological activities. Until recently, substance P (SP) was the only tachykinin known to occur in mammalian species. Substance P-like immunoreactivity (SPLI) has been found in the central nervous system and in sensory neurons innervating many peripheral organs. Now, in addition to SP, mammalian tissues have been found to contain tachykinins related to kassinin and SP; neurokinin A (NKA) and neurokinin B (NKB). The purpose of the present study was to investigate whether pretreatment with capsaicin also decreases the concentration of mammalian tachykinins other than SP in some peripheral organs. The results presented here indicate that capsaicin pretreatment depletes certain tissues of tachykinins other than SP. As has previously been shown for SP, the largest relative depletions were found in the tissues containing a high density of primary sensory afferents (ureters, urinary bladder. These results indicate a close relationship between SP and other mammalian tachykinins such as NKA and NKB, not only in terms of similar tissue distribution, but also in terms of similar reactions to capsaicin, suggesting that NKA and NKB are additional peptides of sensory origin. It is therefore interesting that NKA, but not NKB, has a very potent broncho-constrictor action and causes protein extravasation in the tissues, where acute administration of capsaicin reduced the neurokinin concentrations. Neurokinin A may therefore contribute, together with SP, to the neurogenic inflammatory response.

Theodorsson-Norheim, E., Hua, X., Brodin, E., and *Lundberg, J. M.*

Acta Physiologica Scandinavica 124:129-131, 1985.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, Petrus and Margareta Hedlunds Foundation, Magnus Bergvalls Stiftelse, the Astra Foundation, the Nordisk Insulinfond, and the Karolinska Institute.

From the Department of Clinical Chemistry, Karolinska Hospital, and Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

NON-CHOLINERGIC VASODILATION IN THE TRACHEOBRONCHIAL TREE OF THE CAT INDUCED BY VAGAL NERVE STIMULATION

The tracheobronchial mucosa is extensively vascularized and it also receives a dense parasympathetic and sensory innervation. Chemical or mechanical irritation of the

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

mucosa induces protective reflexes, such as cough and bronchospasm. In addition, the irritation leads to a clinically and experimentally well-known hyperemia and mucosal swelling. The increased vascular permeability to macromolecules seems to be dependent on activation of sensory nerves which release mediators such as substance P. In the present study, the authors have tried to establish the existence of a vagal non-cholinergic vasodilation in the lower airways using the radioactive microsphere technique to measure blood flow in atropinized cats. The experiments were performed in seven male cats. The data presented in this paper establish the existence of a non-cholinergic vasodilation upon vagal nerve stimulation in the tracheobronchial tree using the microsphere technique. In the nasal mucosa it has been possible to elicit non-cholinergic vasodilation in two principally different ways: (1) by parasympathetic preganglionic nerve stimulation, where the vasodilation is abolished by hexamethonium; and (2) by vasodilation, which is hexamethonium resistant and is elicited by antidromic stimulation of sensory nerves using high threshold stimulation parameters (Lundberg 1984). The parasympathetic non-cholinergic vasodilation is probably due to the release of peptides such as VIP and PHI from postganglionic neurons (Lundberg *et al.* 1984a), while the second type is due to antidromic impulse propagation and the release of tachykinins such as substance P (Lundberg 1984).

Martling, C-R., Anggard, A., and *Lundberg, J. M.*

Acta Physiologica Scandinavica 125:343-346, 1985.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, Petrus and Augusta Hedlunds Foundation, and the Karolinska Institute.

From the Departments of Anesthesiology and Oto-Rhino-Laryngology, Karolinska Hospital, and Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

NEUROPEPTIDE Y (NPY) AND SYMPATHETIC CONTROL OF BLOOD FLOW IN ORAL MUCOSA AND DENTAL PULP IN THE CAT

Neuropeptide Y (NPY) immunoreactivity (-IR) was found to be present in perivascular nerves in the cat dental pulp and oral mucosa. Many ganglion cells in the superior cervical ganglion also contained NPY-IR. Ligation of the inferior alveolar or lingual nerves produced an accumulation of NPY-IR in axons proximal to the site of ligation, suggesting an anterograde axonal transport of the peptide. After unilateral sympathectomy the NPY-IR disappeared in the dental pulp and oral mucosa on the ipsilateral side. Reversed phase high performance liquid chromatography showed that the main peak of NPY-like immunoreactivity found in the superior cervical ganglion co-chromatographed with synthetic porcine NPY. Changes in blood flow in dental pulp or oral mucosa were measured indirectly by recording local clearance of ^{125}I during electrical stimulation of the sympathetic nerve or during close intra-arterial infusion of noradrenaline or NPY. All three procedures resulted in a pronouced decrease in local blood flow of a similar magnitude in both tissues. After α-adrenoceptor blockade with phentolamine, the vasoconstrictor effect of noradrenaline was abolished. However, the effect of sympathetic stimulation after phentolamine was only partically reduced (23-54%) and the response to NPY was almost unaffected by the α-receptor blockade. The remaining effect of sympathetic stimulation after phentolamine was abolished by guanethidine. However, the response to NPY was not changed by the latter drug. In conclusion, the vasoconstrictor response in the dental pulp and oral mucosa caused by activation of sympathetic nerves is more resistant to phentolamine than the response

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

induced by infusion of exogenous noradrenaline. Since NPY is probably co-localized with noradrenaline in the sympathetic perivascular nerves and NPY reduces local blood flow, it is proposed that this peptide is involved in sympathetic vascular control in oral tissues.

Edwall, B., Gazelius, B., Fazekas, A., Theodorsson-Norheim, E., and *Lundberg, J. M.*

Acta Physiologica Hungarica 125:253-264, 1985.

Other support: Swedish Medical Research Council; School of Dentistry, Karolinska Institute; Swedish Tobacco Company; Hedlunds Foundation.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

PRE- AND POSTJUNCTIONAL EFFECTS OF NPY ON SYMPATHETIC CONTROL OF RAT FEMORAL ARTERY

Neuropeptide Y (NPY)-like immunoreactivity (LI) has recently been demonstrated in perivascular, noradrenergic (NA), sympathetic neurons. NPY is a potent vasoconstrictor agent *in vivo* while the contractile effects of NPY on isolated blood vessels are variable. For this study, therefore, the authors have analyzed possible pre- and postjunctional effects of NPY in relation to noradrenergic mechanisms in the rat femoral artery. The data presented in this paper show that, except for the vasoconstrictor activity *per se*, NPY enhances the contractile effects of NA on arterial vascular smooth muscle. The NPY effect may be related to changes in the influx of extracellular calcium, since it was absent after nifedipine. The contractile response to transmural nerve stimulation (TNS) was also increased somewhat by NPY. However, in the present study, NPY in the same concentration caused a marked (about 50%) reduction of TNS-evoked ^{3}H-NA efflux. Thus, it is striking that a marked depression of stimulus-evoked ^{3}H-NA secretion can occur simultaneously with an enhanced vascular smooth muscle contraction. This indicates that postjunctional contractile and facilitatory mechanisms of NPY have a more profound effect on sympathetic neurotransmission than a 50% reduction of NA release. Thus, NPY may have an important role in the regulation of sympathetic vascular control.

Lundberg, J. M. et al.

Acta Physiologica Scandinavica 123:511-513, 1985.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, Petrus and Augusta Hedlunds Foundation, the Astra Foundation and the Karolinska Institute.

From the Departments of Pharmacology and Biochemistry, Karolinska Institute, Stockholm, and Department of Clinical Pharmacology, Sahlgren's Hospital, Gothenburg, Sweden.

AMINO ACID UPTAKE BY HUMAN PLACENTA: ALTERATIONS BY NICOTINE AND TOBACCO SMOKE COMPONENTS AND THEIR IMPLICATIONS ON FETAL GROWTH

The fetus of the smoking mother may partially be subjected to amino acid deficits due to the decreased uptake by trophoblast and the transfer of amino acids from trophoblast to fetal circulation.

1002319494

Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

Sastry, B. V. Rama.

In: Caciagli, F., Giacobini, E. and Paoletti, R. (eds.): *Developmental Neuroscience: Physiological, Pharmacological and Clinical Aspects.* Elsevier Science Publishers, Amsterdam, pp. 137-140, 1984.

Other support: U. S. United States Public Health Service and National Institutes of Health.

From the Department of Pharmacology, Vanderbilt University, Nashville, TN.

EFFECTS OF CALCIUM CHANNEL BLOCKING AGENTS ON MEMBRANE MICROVISCOSITY AND CALCIUM IN THE LIVER OF THE CARBON TETRACHLORIDE TREATED RAT

Membrane microviscosity was determined from the polarized fluorescence of diphenylhexatriene in plasma membranes and microsomes prepared from the liver of carbon tetrachloride treated rats. It was greatly depressed between 12 and 24 hr after the administration of the carbon tetrachloride. Depression of microviscosity was also seen in the liposomes which were prepared from these membranes. There were decreases in phospholipid content and phospholipid methyltransferase activity, but these changes did not appear to explain the decreased microviscosity. A large accumulation of calcium occurred in the liver cells between 12 and 24 hr after the administration of carbon tetrachloride. Chlorpromazine, verapamil and nifedipine, when administered prior to the carbon tetrachloride, partially reduced the later accumulation of calcium and reduced the degree of histological damage observed. When these agents were administered 12 hr after the administration of carbon tetrachloride, they did not reduce the subsequent accumulation of calcium. When administered prior to and 7 hr after carbon tetrachloride, they had a small but potentially significant effect on the microviscosity change. It is suggested that at low levels of microviscosity, a critical threshold may exist below which entry of calcium into the cell is poorly controlled and that calcium channel blocking agents may be ineffective if administered at a time when membrane microviscosity is very low. Tissue calcium accumulation was associated with visible cell damage.

Landon, E. J., Jaiswal, R. K., Kaukam, R. J. and *Sastry, B. V. Rama*

Biochemical Pharmacology 33(22):3553-3560, 1984.

Other support: U. S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University, School of Medicine, Nashville, TN.

CHOLINERGIC PROPERTIES OF CHOLINE ETHERS

Effects of nine choline ethers, $(CH_3)_3N^+CH_2CH(R)\text{-}0\text{-}R'$, on the muscarinic and nicotinic receptors of longitudinal smooth muscle of guinea pig ileum were studied to understand the role of electronic/steric factors at the ether-oxygen in stimulating the cholinergic receptors. Their ED_{50}s to cause contraction of the ileum in presence of hexamethonium (37×10^{-6}M) were 2 to 307 times higher than that of acetylcholine (ACh; 2.9×10^{-7}M). The relative maximal effects of 5 ethers (1.20 to 1.34) were higher than that of ACh (1.0), while 4 exhibited lower maximal effects (< 0.71). These ethers exhibited

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

no significant inhibition of choline acetyltransferase and cholinesterase activities from the longitudinal muscle at their ED_{50}s. Hexamethonium significantly increased the ED_{50}s of 5 choline ethers. The ED_{50}s of some of the ethers also were significantly increased by treating the muscle with physostigmine (38.5×10^{-8}M) or physostigmine and hexamethonium. Atropine ($> 1 \times 10^{-6}$M) blocked the contractions induced by these ethers. The steric hinderance caused by the β-methyl and/or O-alkyl groups and the electron density around the ether-oxygen are limiting the muscarinic, as well as nicotinic, potencies of these choline ethers. Choline ethers possessing the beta methyl and O-*n*-propyl, *iso*-propyl and *ter*-butyl groups presumably release ACh at a site causing inhibitory potential through a secondary pathway.

Chaturvedi, A. K. and *Sastry, B. V. Rama.*

Archives Internationales de Pharmacodynamie et de Thérapie 277(1):15-27, 1985.

Other support: U.S. Public Health Service, National Institutes of Health and North Dakota Division of the American Cancer Society.

From the Department of Pharmaceutical Sciences/Toxology, College of Pharmacy, North Dakota State University Fargo, and the Department of Pharmacology, School of Medicine, Vanderbilt University, Nashville, TN.

AUTOREGULATION OF ACETYLCHOLINE RELEASE: ROLE OF SUBSTANCE P AND METHIONINE ENKEPHALIN

An acetylcholine (ACh)-amplification or positive feedback mechanism and a negative feedback mechanism were proposed for release of ACh from cholinergic nerves. The rate of influx of extracellular calcium ions and two endogenous peptides, substance P (SP) and methionine enkephalin (MEK) were also known to regulate ACh release. Therefore, these studies were undertaken to delineate the various components of the above feedback systems.

Mouse cerebral slices were incubated in a Krebs Ringer bicarbonate buffer containing (methyl-^{3}H) choline (0.1 mM; 0.25 Ci/ml) for 60 min. They were filtered, washed, and transferred to a microbath set up for superfusion with the above buffer. The release of ^{3}H-ACh, SP and MEK into the superfusate was measured as described elsewhere. The following results were observed: (a) The rate of ACh release increased initially for the first 5 min and then declined exponentially. The highest rate of release for SP was observed with 2 min preceding the peak release of ACh. A broad peak for the release of MEK followed that of ACh. (b) SP (0.6 μM) increased ACh release (35%) and Ca^{++} uptake (154%). The long acting enkephalin (D-ala-enkephalinamide, 34 nM) decreased the release of ACh (65%) and well as Ca^{++} uptake (48%). (c) 5-Hydroxymethylfurfuryltrimethylammonium (1.9 nM) decreased ACh release by 50% in a medium containing Ca^{++} (2.6 mM). It blocked ACh release induced by K^+ (20 mM) and disteroylphosphatidic acid (43 μM). It increased both spontaneous (14 times) and evoked (2-3 times) release of SP. It decreased spontaneous (16 times) and evoked (20 times) release of MEK. These results suggest that the positive feedback for ACh release may operate through a muscarinic receptor, SP and activation of Ca^{++} influx, and the negative feedback through a muscarinic receptor, MEK and inhibition of Ca^{++} influx.

Sastry, B. V. Rama. Jaiswal, N., and Tayeb, O. S.

In: *Substance P Metabolism and Biological Actions*, Jordan, C.C. and Oehme, P. (eds.): Taylor and Francis, London, 1985, p. 243.

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

Other support: U. S Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

NICOTINE

The mediation of the behavioral effects associated with nicotine and smoking has been examined in humans and in several animal species. In general, an improvement in behavior or a reduction in stress has been reported. Since metabolism and dosages have been shown to depend on the species examined and on the rate of administration, it is difficult to speculate on the processes in humans that are influenced by nicotine. Aside from the possible addictive properties of nicotine, its behavioral effects suggest that nicotine may function as a neurotransmitter. The action of nicotine on the nervous system may be mediated through a specific binding site as yet poorly identified. The nicotine binding site in brain probably differs from the classical nicotinic cholinergic receptor site as described in the peripheral nervous system, since binding studies with labeled acetylcholine, α-bungarotoxin, or nicotine suggest that these ligands bind to different sites in brain. Nicotine is of particular interest because of its wide use by man, because it is among the compounds that penetrate the brain very rapidly, and because it affects behavior.

Sershen, H.

In: Lajtha, A. (ed.): *Handbook of Neurochemistry, vol.* 9, New York: Plenum Publishing Corporation, 1985, pp. 263-278.

From the Center for Neurochemistry, Nathan S. Kline Research Institute, Ward's Island, New York.

PROTECTION AGAINST 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE NEUROTOXICITY BY THE ANTIOXIDANT ASCORBIC ACID

Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; 2 x 8 mg/kg retro-orbital) to BALB/cBy mice reduced [^{3}H]mazindol binding to striatal membranes by 50%. Reactive oxygen derivatives have been suggested to be involved in MPTP neurotoxicity; therefore, these researchers examined the effects of ascorbic acid (an antioxidant). Ascorbic acid (100 mg/kg) given 20 min prior to MPTP administration appreciably prevented the reduction of [^{3}H]mazindol binding. The involvement of oxidative processes in the mechanism of MPTP neurotoxicity may suggest a relationship to the etiology of Parkinson's disease and the possible benefit of treatment with ascorbic acid.

Sershen, H. et al.

Neuropharmacology 24(12):1257-1259, 1985.

From the Center for Neurochemistry, Nathan S. Kline Research Institute, Ward's Island, New York.

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

COMPARISON OF [^{3}H]NICOTINE AND [^{3}H]ACETYLCHOLINE BINDING IN MOUSE BRAIN: REGIONAL DISTRIBUTION

In a continuing study of nicotine binding sites, we determined the relative amount of nicotine binding and acetylcholine binding in various brain regions of C57/BL and of DBA mice. Although midbrain showed the highest and cerebellum the lowest binding for both [^{3}H]nicotine and [^{3}H]acetylcholine, the ratio of nicotine to acetylcholine binding showed a three-fold regional variation. Acetylcholine inhibition of [^{3}H]nicotine binding indicated that a portion of nicotine binding was not inhibited by acetylcholine. These results indicate important differences between the binding of (±)-[^{3}H]nicotine and that of [^{3}H]acetylcholine.

Sershen, H., Reith, M. E. A., Hashim, A., and Lajtha, A.

Research Communications in Chemical Pathology and Pharmacology **48**(3)345-352, 1985.

From the Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island, New York.

EFFECT OF N-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) ON AGE-RELATED CHANGES IN DOPAMINE TURNOVER AND TRANSPORTER FUNCTION IN THE MOUSE STRIATUM

Damage to neuronal cells, either during the normal aging process or related to MPTP neurotoxicity, have implicated oxidative free radical mechanisms. Glutathione (a natural reducing agent) is significantly lower in the substantia nigra compared to other brain regions and virtually absent in the nigra of Parkinsonian patients (Perry *et al.*, 1982). If MPTP neurotoxicity involves free radical mediated processes, it was of interest to examine MPTP neurotoxicity in the aged mouse, in which antioxidant mechanisms may be comprised. The present study shows that in MPTP treated animals, [^{3}H]mazindol binding was decreased by 24% more in aged mice as compared to young mice, and a similar increased sensitivity to the neurotoxic effects of MPTP was observed in a decreased dopamine level (26% lower in aged MPTP treated animals versus young MPTP treated animals). Altogether the data indicate MPTP administration to mice results in a critical loss of dopaminergic cells (40-64%), necessitating both an increase in synthesis and in release (turnover) of dopamine from the surviving neurons. MPTP neurotoxicity is greater in the older mouse possibly associated with an age related increase in the susceptibility of dopaminergic cells to oxidative damage; indicating that aged mice can be useful in studying mechanisms of MPTP neurotoxicity.

Sershen, H., Mason, M. F., Hashim, A., and Lajtha, A.

European Journal of Pharmacology **113**:135-136, 1095.

From the Center for Neurochemistry, Nathan S. Kline Institute, Ward's Island, New York.

ACTH PEPTIDES AS ORGANIZERS OF NEURONAL PATTERNS IN DEVELOPMENT; MATURATION OF THE RAT NEUROMUSCULAR JUNCTION AS SEEN BY SCANNING ELECTRON MICROSCOPY

SEM was used to visualize the normal postnatal development of the neonatal rat neuromuscular junction (nmj). Maturational changes evoked by ACTH/MSH 4-10 (10 μg/kg/day IP) or ACTH/MSH 4-9 (Org 2766) (0.01 μg/kg/day IP) were compared to

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controls and to pups treated with nicotine during prenatal and postnatal life, or only during the gestation period. Pregnant females received 0.25 mg/kg 2x daily IP; neonates 0.05 mg/kg/day SC. The Desaki and Uehara and Fahim *et al.* methods revealed the nmj on the extensor digitorum muscle to be covered by a delicate drapery of postjunctional folds that surround the immature endplate region. By the second week of postnatal life, these folds become more complex and cover a larger area. Upon maturation the folds descend and invaginate into the muscle fiber. Peptide treatment with either ACTH/MSH 4-10 or ACTH/MSH 4-9 accelerates maturation of the endplate as demonstrated by the increased convolutions of the folds. Similar effects follow nicotine administration. The observed changes in morphology of the developing nmj subjected to nicotine may be mediated through nicotine-evoked ACTH release.

Frischer, R. E., El-Kawa, N. M. and *Strand, F. L.*

Peptides 6(2),13-19, 1985.

Other support: Biomedical Research Support, Grant Program Division of Research Resources, and National Institutes of Health.

From the Biology Department, New York University, New York.

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SPECIFIC BINDING AND METABOLISM OF (-)- AND (+)- [^{3}H]-NICOTINE IN ISOLATED RAT HEPATOCYTES AND HEPATOCYTE MEMBRANES

The specific binding of (-)- and (+)- ^{3}H-nicotine, as well as their relative rates of metabolism, were investigated in isolated rat hepatocytes and hepatocyte membranes. A Scatchard plot of both tritiated enantiomers revealed two components, with the higher affinity site having K_d values of 2×10^{-10} and 3×10^{-9} M and B_{max} values of 5×10^{-15} and 4×10^{-16} moles/mg protein for the (-)- and (+)-enantiomers, respectively. The lower affinity site for both enantiomers had a K_d of 4×10^{-9} M and B_{max} of 5×10^{-14} moles/mg protein. The pH optimum of binding was in the higher pH range, in contrast to brain membranes where the optimum was 6.5 involving the protoneated form of nicotine. A good correlation was observed between the pharmacologic potency of a group of nicotine analogues and their ability to bind to intact and hepatocyte membranes. The rate of conversion of nicotine to cotinine in hepatocytes is related to the degree of binding and accumulation. The results are discussed in terms of the possible relationship of the nicotine binding and translocation to its metabolism.

*Abood, L.G. et al.

Archives internationales de Pharmacodynamie et de Therapie 273(1):62-73, 1985.

Other support: DA00464.

From the Center for Brain Research and Department of Biochemistry, University of Rochester Medical Center, Rochester, NY.

EFFECTS OF CHRONICALLY ADMINISTERED NICOTINE AND SALINE ON MOTOR ACTIVITY IN RATS

This study investigated the differential effects of chronically administered nicotine and saline on motor activity on the rat. Nicotine was administered via a subcutaneously implanted osmotic minipump to effect an 8 hour off, 16 hour on flow. Subjects were 48 male and 48 female albino rats, each about 165 days old. Activity was monitored every hour for 192 consecutive hours. Results indicated that the female animals were more active than the males and that animals receiving nicotine were significantly more active on the first two days of drug administration than control animals; however, by the fourth day there were no significant differences between the activity levels of animals that received nicotine and those of control animals.

Cronan, T., Conrad, J., and *Bryson, R.*

Pharmacology Biochemistry & Behavior 22(5):897-899, 1985.

From the Department of Psychology, San Diego State University, San Diego, CA.

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

CHARACTERIZATION OF ANTIBODIES TO NICOTINE

The researchers studied the specificity of antibodies produced against four different hapten-carrier conjugates: (D,L)-6-(p-aminobenzamido) nicotine-bovine serum albumin (BSA), (D,L)-6-(ε-aminocaparamido) nicotine-BSA, (D,L)-2-(p-aminobenzamido) nicotine-BSA and (D,L)-6-aminonicotine BSA. All of the produced antibodies showed about equal specificity for both D- and L-isomers of nicotine. The carbon length of the spacing molecule between the hapten and carrier molecule did not given substantial influence to the specificity for the pyrolidine portion of the nicotine molecule. The specificity on pyridine portion was increased as the length of the spacing molecule increased. All of the produced antibodies did not cross-react significantly (less that 0.1%) with cotinine, N-methyl pyridinium compounds of (L)-N'nitrosonornicotine. Studies on nicotine levels in the biological fluids of man and rabbits indicated that the radioimmunoassay technique developed in the present studies could detect as low as 2-5 ng/ml.

Castro, A., Monji, N., Ali, H., Bowman, E. R., and McKennis, H.

Biochemical Archives 1(4)173-183, 1985.

From the Department of Pathology, Hormone Research Laboratory, University of Miami School of Medicine, Miami, FL ,and the Division of Biochemical Pharmacology, Medical College of Virginia, Richmond.

"SEMI-RIGID" AND "FLEXIBLE" LINKAGES IN ANTIBODY PRODUCTION FOR DETERMINATION OF NICOTINE

An increasing interest in precise methods for determination of nicotine levels in body fluids has led to development of numerous procedures, all of which have some disadvantages. However, radioimmunoassays and related procedures have proven to have certain unique advantages with small samples and low nicotine concentrations. In the first published study of a radioimmunoassay for determination of nicotine, it was concluded that 2- and 6-aminonicotine were not suitable functionalized nicotine haptens for various reasons. In the present study of the use of racemic aminonicotine as a functionalized hapten, nicotine antibodies suitable for use in nicotine determinations have been produced from antigens in which both "flexible" and "semi-rigid" chains serve to couple racemic 6-aminonicotine to bovine serum albumin.

Castro, A., McKennis, H., Jr., Monji, N., and Bowman, E. R.

Biochemical Archives 1(4):205-214, 1985.

Other support: The American Tobacco Company.

From the Department of Pathology, Hormone Research Laboratory, University of Miami School of Medicine, Miami, FL, and the Division of Biochemical Pharmacology, Medical College of Virginia, Richmond.

PHOSPHORYLATION OF A CHROMAFFIN GRANULE-BINDING PROTEIN BY PROTEIN KINASE C

Protein kinase C was detected in a group of Ca^{2+}-dependent chromaffin granule membrane-binding proteins (chromobindins) on the basis of Ca^{2+}-, phosphatidylserine-,

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

1,2-diolein-, and phorbol myristate acetate-stimulated histone kinase activity. When the chromobindins were incubated with [γ-^{32}P]ATP, calcium, and phosphatidylserine, ^{32}P was incorporated predominantly into a protein of mass 37 ± 1 kilodaltons (chromobindin 9, or CB9). Phosphorylation of this protein was also stimulated by diolein and phorbol myristate acetate, indicating that it is a substrate for the protein kinase C activity present in the chromobindins. Maximum phosphate incorporation into CB9 in the presence of 1mM calcium, 75 μg/ml of phosphatidylserine, 2.5 μg/ml of diolein, and 12.5 μg/ml of dithiothreitol was 0.53 mol/mol of CB9 in 5 min. Eight ^{32}P-labeled phosphopeptides were resolved in two-dimensional electrophoretic maps of trypsin digests of CB9. Phosphoamino acid analysis revealed that phosphorylation was exclusively on serine (94%) and threonine (6%) residues. Incubation of the chromobindins with chromaffin granule membranes in the presence of [γ^{32}P]ATP resulted in the incorporation of ^{32}P into eight additional proteins besides CB9 that could be separated from the membranes by centrifugation in the presence of ethylene glycol bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid. We suggest that phosphorylation of CB9 or these additional eight proteins may regulate events underlying exocytosis in the chromaffin cell.

Summers, T. A. and *Creutz, C. E.*

The Journal of Biological Chemistry 260(4):2437-2443, 1985.

Other support: National Institutes of Health, National Science Foundation and University of Virginia Diabetes Research and Training Center.

From the Department of Pharmacology, University of Virginia, Charlottesville.

PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C ACTIVITY OF CHROMAFFIN GRANULE-BINDING PROTEINS.

Using [U-^{14}C] phosphatidylinositol as substrate, Ca^{2+}-dependent phospholipase C activity was detected in a group of bovine adrenal medullary proteins that bind to chromaffin granule membranes in the presence of Ca^{2+} ("chromobindins," Creutz, C.E., Dowling, L.G., Sando, J.J., Villar-Palasi, C., Whipple, J.H., and Zaks, W.J. (1983) *J. Biol. Chem.* 258, 14664-14674). The activity was maximal at neutral pH and represented an 80- to 240-fold enrichment of adrenal medullary cytosol phospholipase C activity measured at pH 7.3. The stimulation of activity by Ca^{2+} was complex; no activity was present in the absence of calcium, 25% activation occurred at 1 μM Ca^{2+}, and full activation at 5 mM Ca^{2+}. The enzyme bound to chromaffin granule membranes in the presence of 2 mM Ca^{2+} but was released at 40 μM, suggesting that intrinsic enzyme activity may be regulated by [Ca^{2+}] at 1 μM but additional activation at higher concentrations of calcium is seen *in vitro* as a result of Ca^{2+}-dependent binding of the active enzyme to substrate-containing membranes. This enzyme may generate diacylglycerol and phosphorylated inositol to act as intracellular messengers in the vicinity of the chromaffin granule membrane during the process of exocytosis.

Creutz, C. E., Dowling, L, Kyger, E., and Franson, R. C.

The Journal of Biological Chemistry 250(12):7171-7173, 1985.

Other support: National Institutes of Health and National Science Foundation.

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From the Department of Pharmacology, University of Virginia, Charlottesville, and the Department of Biochemistry, Medical College of Virginia, Richmond

DIRECT EFFECTS OF ADRENOCORTICOTROPIC HORMONE ON BOVINE ADRENOMEDULLARY CELLS: ADENOSINE 3',5'-MONOPHOSPHATE-DEPENDENT PHOSPHORYLATION OF TYROSINE HYDROXYLASE

Release of adrenal catecholamine by carbachol has been shown to coincide with an increase in intracellular cAMP levels. Bovine adrenal medullary (BAM) cells were prepared and maintained in culture and used to examine the role of cAMP in stimulus-secretion coupling. The addition of ACTH to these cells caused a 10- to 50-fold increase in cellular cAMP without an effect on catecholamine secretion, suggesting cortical cell contamination. Percoll density separation of both BAM cells and adrenal cortical cells revealed that the greatest cAMP responses to ACTH corresponded to the catecholamine-containing cell fractions and not to those density layers where cortical cells sedimented.

BAM cells isolated on Percoll did not metabolize [^{14}C]cholesterol to steroids as would be expected were the ACTH-stimulated cAMP accumulations due to cortical cell contamination of the cultures. ACTH stimulated protein phosphphorylation in ^{32}P-labeled BAM cells in a manner indistinguishable from that induced by carbachol and forskolin. The major soluble phosphoprotein to be affected by these agents had a relative mol wt of 55-57 kdaltons on sodium dodecyl sulfate-gels and corresponded to tyrosine hydroxylase, which is a specific marker enzyme in the adrenal for chromaffin cells.

We propose that bovine adrenal chromaffin cells express ACTH receptors which are coupled to adenylate cyclase. While no acute effect of ACTH was found on catecholamine secretion, ACTH may play a direct role in the regulation of catecholamine synthesis by stimulating the phosphorylation of tyrosine hydroxylase by cAMP-dependent protein kinase.

Michener, M. L., Peach, M. J., and *Creutz, C. E.*

Endocrinology 117(2):730-737, 1985.

Other support: National Institutes of Health, and the National Science Foundation with additional assistance from the University of Virginia Diabetes Research and Training Center and Vascular Smooth Muscle Program Project.

From the Department of Pharmacology, University of Virginia, Charlottesville.

[illegible] OH SCAVENGER IN AQUEOUS SOLUTIONS AND IN BIOLOGICAL SYSTEMS

By using the technique of pulse radiolysis to generate OH radicals, the authors have determined through competition with SCN^-, I^- and $Fe(CN)_6^{4-}$ the reaction rate constant of mannitol with OH radical at pH = 7 to be $(1.8 \pm 0.4) \times 10^9\ M^{-1}\ s^{-1}$. This value justifies the use of mannitol as an OH scavenger. However, in biological systems there is some uncertainty concerning the relevant concentration of this scavenger. The concentration inside the cell may be very different from that in the solution because of active transport on the one hand or through metabolism on the other. Even if the authors can determine the concentration of mannitol inside the cell, they are not able to determine the concentration of the targets where the damage occurs. For all these

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reasons they cannot expect that the effect of OH scavengers in biological systems will be proportional to their rate constants with OH.

Goldstein, S. and *Czapski, G.*

International Journal of Radiation Biology 46(6):725-729, 1984.

Other support: U.S. Department of Energy, and Gesellschaft fur Strahlen Forschung, Neuherberg, West Germany.

From the Department of Physical Chemistry, Hebrew University of Jerusalem, Jerusalem, Israel.

KINETICS OF OXIDATION OF CUPROUS COMPLEXES OF SUBSTITUTED PHENANTHROLINE AND 2,2'-BIPYRIDYL BY MOLECULAR OXYGEN AND BY HYDROGEN PEROXIDE IN AQUEOUS SOLUTION

The kinetics and the reaction mechanism of copper(I) complexes of 5-methyl-1,10-phenanthroline, 5-chloro-1,10-phenanthroline, 5-nitro-1,10-phenanthroline, 2,9-dimethyl-1,10-phenanthroline, and 2,2'-bipyridyl with oxygen and hydrogen peroxide have been investigated in aqueous solutions with use of the pulse radiolysis technique. The oxidation by O_2 is second order in the copper(I) complex, while the oxidation by H_2O_2 is first order in the copper(I) complex. Both reactions are first order in oxidants. The kinetic results of the oxidation of copper(I) complexes by oxygen are interpreted by a mechanism that proceeds via a superoxide intermediate.

Goldstein, S. and *Czapski, G.*

Inorganic Chemistry 24(7):1087-1092, 1985.

Other support: Gesellschaft für Strahlen Forschung, Neuherberg and the U.S. Department of Energy.

From the Department of Physical Chemistry, Hebrew University of Jerusalem, Jerusalem, Israel.

THE CONTRIBUTION OF ENDOGENOUS AND EXOGENOUS EFFECTS TO RADIATION-INDUCED DAMAGE IN THE BACTERIAL SPORE

Radical scavengers such as polyethylene glycol 400 and 4000 and bovine albumin have been used to define the contribution of *exogenous* and *endogenous* effects to the gamma-radiation-induced damage in aqueous bacterial suspensions of *Bacillus pumilus* spores. The results indicate that this damage in the bacterial spore is predominantly *endogenous* both in the presence of 1 atmosphere of oxygen and in anoxia.

Jacobs, G. P., Samuni, A., and *Czapski, G.*

International Journal of Radiation Biology 47(6):621-627, 1985.

Other support: U. S. Department of Energy.

From the Department of Pharmacy, School of Pharmacy, Department of Molecular Biology, School of Medicine, and Department of Physical Chemistry, Hebrew University of Jerusalem, Jerusalem, Israel.

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EFFECTS OF ETHANOL AND TEMPERATURE ON GLUCOSE UTILIZATION IN THE IN VIVO AND ISOLATED PERFUSED MOUSE BRAIN

A [^{14}C]2-deoxy-D-glucose technique was used to investigate the effects of ethanol and temperature variation on the rate of glucose utilization in the isolated perfused mouse brain. Glucose utilization rates in six regions of the isolated perfused mouse brain at 37°C were similar to the rates in comparable regions of the *in vivo* mouse brain. The temperature dependence of the glucose utilization rate in the perfused mouse brain suggests that a change in the rate-limiting step of cerebral glucose metabolism occurred between 28°C and 32°C. To investigate the possible involvement of temperature-dependent membrane phase transitions, brain perfusions were performed with a medium containing ethanol, which is known to increase membrane fluidity. Perfusion of the mouse brain with a fluid containing 87 mM ethanol (400 mg/dl) resulted in decreases in the glucose utilization rates at 37 but not at 28°C. At 28°C, ethanol produced an increase in glucose utilization in the medulla-pons and in the cerebellum of perfused brains. *In vivo* rates of cerebral glucose utilization in two lines of mice selectively bred for differences in ethanol-induced sleep time indicated that no differences in basal metabolism or following a 4-g/kg dose of ethanol existed between the two lines. Interpretations of ethanol's differential effects on cerebral glucose utilization rates in the isolated perfused mouse brain at 28°C and 37°C are presented along with a discussion of the apparent discrepancies observed when *in vivo* and perfused brains are treated with equivalent concentrations of ethanol.

Towell, J. F., III, and *Erwin, V. G.*

Alcoholism: Clinical and Experimental Research 6(1):110-116, 1982.

Other support: U. S. Public Health Service.

From the Alcohol Research Center, School of Pharmacy, University of Colorado, Boulder.

EFFECTS OF NICOTINE ON β-ENDORPHIN, α MSH, AND ACTH SECRETION BY ISOLATED PERFUSED MOUSE BRAINS AND PITUITARY GLANDS, *IN VITRO*

The effects of nicotine on secretion of the pituitary peptides β-endorphin, α MSH and ACTH were studied using the isolated perfused mouse brain (IPMB) and isolated superfused pituitaries of C3H mice. Nicotine (6.1 μM) stimulated secretion of β-endorphin immunoreactivity from C3H IPMB approximately twofold. Secretion of α MSH immunoreactivity was stimulated approximately two- and sixfold by 6.1 μM and 12.2 μM nicotine, respectively. However, nicotine (6.1 μM) had no direct effect on the secretion of β-endorphin, α MSH, or ACTH immunoreactivities from the isolated superfused pituitaries. The data suggest nicotine acts in the brain to stimulate pituitary secretion of α MSH and β-endorphin. Electrocorticographic (ECoG) activity of the IPMB was monitored. Nicotine induced characteristic ECoG changes including a reduction of input voltage, a biphasic response of rapid desynchronization followed by prolonged synchronization, and seizure at high doses (12.2 μM).

Marty, M. A., *Erwin, V. G.*, Cornell, K., and Zgombick, J. M.

Pharmacology, Biochemistry & Behavior 22:317-325, 1985.

Other support: U. S. Public Health Service.

From the Center for Alcohol Research, School of Pharmacy, University of Colorado, Boulder.

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

VANADATE ENHANCEMENT OF THE OXIDATION OF NADH BY $O_2^{\cdot-}$: EFFECTS OF PHOSPHATE AND CHELATING AGENTS.

Vanadate markedly stimulates the oxidation of NADH by $O_2^{\cdot-}$. Both phosphate and Tris are inhibitory, but phosphate diminishes the greater inhibitory effect of Tris and thus gives the appearance of stimulating when added to Tris-buffered reaction mixtures. Chelating agents moderately increased the oxidation of NADH but eliminated the much greater catalytic effect of vanadate. Desferal was the most effective of the chelating agents, and could be used to titrate vanadate spectrophotometrically or in terms of the diminution of its catalytic activity. This permitted the demonstration that metavanadate or orthovanadate could form 1:1 complexes with desferal and that orthovanadate was the catalytically active species.

Darr, D. and *Fridovich, I.*

Archives of Biochemistry and Biophysics 243(1):220-227, 1985.

Other support: United States Army Research Office, National Science Foundation, Glenn Foundation, and National Institutes of Health.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

ESSENTIALITY OF THE ACTIVE-SITE ARGININE RESIDUE FOR THE NORMAL CATALYTIC ACTIVITY OF Cu,Zn SUPEROXIDE DISMUTASE.

Chemical modification of bovine and yeast Cu,Zn superoxide dismutases with phenylglyoxal diminishes the catalytic activities by ≥ 98% and treatment of these enzymes with butanedione plus borate leads to ≥ 96% inactivation. The activity loss is accompanied by the modification of less than two arginine residues per subunit with no concomitant loss of Cu or Zn. The phenylglyoxal-modified enzymes require at least a 20-fold greater concentration of cyanide for 50% inhibition than do the corresponding native enzymes. Polyacrylamide-gel electrophoresis and activity staining of the phenylglyoxal-inactivated enzymes demonstrate that the residual activity is largely associated with modified forms that bear lower net positive charge than the native superoxide dismutases.

Borders, C. L., Jr., Saunders, J. E., Blech, D. M., and *Fridovich, I.*

Biochemistry Journal 230:771-776, 85.

Other support: Petroleum Research Fund, U. S. Army Research Office, the Natic.. Science Foundation, and the Glenn Foundation.

From the Department of Chemistry, College of Wooster, Wooster, OH, and the Department of Biochemistry, Duke University Medical Center, Durham, NC.

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PSEUDOCATALASE FROM *LACTOBACILLUS PLANTARUM*: EVIDENCE FOR A HOMOPENTAMERIC STRUCTURE CONTAINING TWO ATOMS OF MANGANESE PER SUBUNIT

An improved procedure for the isolation of the pseudocatalase of *Lactobacillus plantarum* has been devised, and the quaternary structure and manganese content of this enzyme have been reexamined. Sedimentation equilibrium of the native enzyme at several salt concentrations gave a molecular weight of 172,000. The subunit weight, obtained by sedimentation equilibrium in 6.4M guanidinium chloride, with or without prior reduction and carboxymethylation, was 34 kilodaltons. The amino acid composition indicated 150 Arg + Lys, and after exhaustive tryptic digestion, 32 peptides were resolved. These data suggest that the pseudocatalase is a homopentamer. Cross-linking with dimethyl suberimidate, followed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, yielded five major bands, another indication of pentameric structure. The manganese content was found to be 1.8-2.4 per subunit. $s^0_{20,w}$ was found to be 9.6S and $f/f_0 = 1.2$, suggesting a globular structure of Stokes radius 44 Å.

Beyer, W. F., Jr. and *Fridovich, I.*

Biochemistry **24**(23):6460-6467, 1985.

Other support: National Institutes of Health, U. S. Army Research Office, and the National Science Foundation.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

THE SALMONELLA/MAMMALIAN MICROSOME MUTAGENICITY TEST: COMPARISON OF HUMAN AND RAT LIVERS AS ACTIVATING SYSTEMS

The mutagenicity of several test compounds was verified by the Salmonella/microsome mutagenicity test (Ames test), using both human liver and rat liver (untreated or pretreated with Aroclor 1254) S9 under identical experimental conditions. Aflatoxin B_1, 3-methylcholanthrene, and cigarette-smoke condensate were less mutagenic in the presence of human-liver S9 than in the presence of rat-liver S9 (particularly after treatment with Aroclor 1254). The opposite was observed with 2-aminoanthracene and to a lesser degree with 2-aminofluorene; correlation studies indicate that the two compounds were activated by the same or by very similar enzymes, probably cytochrome P-450s. These results clearly indicate that human-liver S9, as an activating system, behaves differently than rat-liver S9, therefore, it may constitute a useful, additional tool for the study of mutagenicity and, probably, carcinogenicity in man.

Beaune, P., Lemestre-Gornet, R., Kremers, P., Albert, A., and *Gielen, J.*

Mutation Research **156**:139-146, 1985.

Other support: INSERM (France's National Institute of Health and Medical Research).

From the Laboratoire de Chimie Medicale, Institut de Pathologie, Universite de Liege, Liege, Belgium.

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MONOCLONAL ANTIBODIES AGAINST HUMAN LIVER CYTOCHROME P-450

Monoclonal hybridomas which produce antibodies against human liver microsomal cytochrome P-450 were developed. Three similar hybridomas produced antibodies which recognized an epitope specific to a family of human P-450 isozymes (P-450$_6$). This epitope was also present on cytochrome P-450 PCN-E (pregnenolone-16α-carbonitrile induced) from rat liver microsomes, but this isozyme differed from the human P-450$_6$ by its molecular weight. These antibodies enabled the authors to quantify cytochrome P-450$_6$ in human liver microsomes and to demonstrate an important quantitative polymorphism in the human liver monooxygenase system.

Beaune, P., Kremers, P., Letawe-Goujon, R., and *Gielen, J. E.*

Biochemical Pharmacology **34**(19):3547-3552, 1985.

Other support: INSERM (France's National Institute of Health and Medical Research).

From the INSERM, Faculte de Medicine Necker, Paris, France.

EVIDENCE FOR REGULATION OF ACTIN SYNTHESIS IN CYTOCHALASIN D-TREATED HEp-2 CELLS

In HEp-2 cells treated with 0.2 or 2.0 μM cytochalasin D (CD), the relative rate of actin synthesis increased for about 12h and then reached a plateau; this increase was suppressed by actinomycin D (AD). When CD was washed from cells which had been treated for 20 h, the elevated rate of actin synthesis declined to the control value within ca 4 h, as the actin-containing cytoskeletal components rearranged by CD recovered their normal morphology. Subsequently, actin synthesis was depressed below control values for a prolonged period; during recovery from 2 h treatment with CD, this depression was of much shorter duration. Re-addition of CD to cells after a 3h recovery period again induced the cytoskeletal alterations characteristic of CD treatment but did not reverse the prior decline in the rate of actin synthesis. In HEp-2 cells treated with cycloheximide during exposure to CD for 20h, the relative rate of actin synthesis measured after removal of cycloheximide was twofold higher than with CD alone and such cells exhibited a twofold slower decline in the rate of actin synthesis during recovery from CD in the continued presence of cycloheximide. These effects of cycloheximide, which resemble observations on "super-induction," suggest that actin synthesis in CD-treated and recovering HEp-2 cells may be regulated by a repressor protein. The possibility that the proposed repressor protein is actin and that actin may thus be a feedback inhibitor of its own synthesis is discussed.

Tannenbaum, J., Brett, J. G. and *Godman, G. C.*

Experimental Cell Research 160:435-448, 1985.

Other support: Muscular Dystrophy Association and National Institutes of Health.

From the National Science Foundation and the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

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MACROVACUOLATION INDUCED BY CYTOCHALASIN: ITS RELATION TO THE CYTOSKELETON, MORPHOLOGICAL AND CYTOCHEMICAL OBSERVATIONS

At higher doses of cytochalasin (e.g. 3 μg/ml for 3-20 hr), cells of the rat fibroblastoid line, Hmf, undergo extreme retraction, arborization, and subsequent rounding, and develop big cystic vacuoles. These vacuoles are always closely invested by microfilamentous masses, the CD-induced derivatives of the actin-based cytoskeleton, which aggregate in the endoplasm. Vacuolation is progressive (e.g. 12% cells at 6 hr, > 80% at 18 hr), related to total dose (concentration × time) and to congener (CD > CB). Vacuole membranes have the same dimension (85 Å), surface marker 5'nucleotidase, and junctional specializations as those found at the cell surface, they lack the membrane markers associated with endomembrane systems (e.g. AcPase, TPPase, IDPase) and are not lysosomal. Vacuoles represent internalized plasma membrane, they apparently result from retention in the endoplasm, and fusion, of pinocytotic vesicles originating at the cell surface. Vacuole membrane is always in intimate relation to the actin-based microfilament aggregates that surround the vacuoles, and actin-membrane linker proteins fodrin and vinculin are localized at the vacuole boundaries. Vacuoles and their enveloping actin-filament aggregates are surrounded by arrays of vimentin-based intermediate filaments. A new membranous compartment with characteristics of plasma membrane is thus formed within the cell under the influence of CD. Rounding brought about by other means causes no vacuolization. Macrovacuolation, like the other changes caused by CD, is completely reversible on restoration of cells to normal medium.

Brett, J. G. and *Godman, G. C.*

Tissue & Cell 16(3):311-324, 1984.

Other support: National Institutes of Health.

From the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

MONOCLONAL ANTIBODIES DETECT THE CONSERVATION OF MUSCARINIC CHOLINERGIC RECEPTOR STRUCTURE FROM *DROSOPHILA* TO HUMAN BRAIN AND DETECT POSSIBLE STRUCTURAL HOMOLOGY WITH α_1-ADRENERGIC RECEPTORS

Muscarinic cholinergic receptors isolated from *Drosophila* heads, rat and human brain, dog heart, and monkey ciliary muscle were examined for structural similarities/differences by utilizing isoelectric focusing, sodium dodecyl sulfate/polyacrylamide gel electrophoresis, and monoclonal antibody cross-reactivity. Muscarinic receptors were affinity labeled with [^{3}H]propylbenzilylcholine mustard and subjected to isoelectric focusing. Muscarinic receptors from each species focused with an isoelectric point of 5.9. The same proteins all migrated with an apparent molecular mass of 80,000 daltons on sodium dodecyl sulfate gels. Six hybridomas secreting monoclonal antibodies specific for muscarinic receptors were developed by using purified rat brain muscarinic receptors as the antigen. The six different monoclonal antibodies immunoprecipitated muscarinic receptors from all tissues and species tested, including human and *Drosophila* brains, with equal efficacy. These data indicate that muscarinic receptors are highly conserved over a considerable evolutionary period. One of the six muscarinic receptor monoclonal antibodies also immunoprecipitated rat liver α_1-adrenergic receptors. Furthermore, two out of five monoclonal antibodies raised

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against those receptors immunoprecipitated muscarinic receptors. These data suggest that some degree of structural homology exists between muscarinic cholinergic receptors and α_1-adrenergic receptors.

Venter, J.C., Eddy, B., *Hall, L.M.*, and Fraser, C.M.

Proceedings of the National Academy of Sciences of the United States of America 81:272-276, 1984.

Other support: American Heart Association.

From the Department of Molecular Immunology, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, and the Department of Genetics, Albert Einstein College of Medicine, The Bronx, NY.

α-BUNGAROTOXIN BLOCKS EXCITATORY SYNAPTIC TRANSMISSION BETWEEN CERCAL SENSORY NEURONES AND GIANT INTERNEURONE 2 OF THE COCKROACH, *PERIPLANETA AMERICANA*

1. Autoradiographic localization of an ^{125}I-α-bungarotoxin binding component revealed that specific binding was distributed mainly in the neuropile and to some extent in the periphery of the terminal abdominal ganglion of the cockroach, *Periplaneta americana*.

2. Action potentials recorded from the axon of GI 2 under current-clamp conditions were not affected by exposure to 1.0×10^{-5}M α-bungarotoxin.

3. Excitatory postsynaptic potentials recorded from GI 2, evoked by stimulation of cercal sensory neurones, were sensitive to block by relatively low (from 10^{-9} to 10^{-7}M) concentrations of α-bungarotoxin. The time-course for blockade was found to depend on toxin concentration and frequency of afferent stimulation.

4. Excitatory postsynaptic potentials recorded from GI 2 were not affected by exposure to 1.0×10^{-6}M quinuclidinyl benzilate.

5. We conclude that at least a portion of the ^{125}I-α-bungarotoxin binding component represents cholinergic receptors which have a postsynaptic function at synapses between cercal sensory neurones and GI 2.

Sattelle, D.B., Harrow, I.D., Hue, B., Pelhate, M., Gepner, J.I., and *Hall, L.M.*

Journal of Experimental Biology 107:473-489, 1983.

Other support: Ciba Foundation Anglo-French Exchange Bursary and the U.S. National Science Foundation.

From the A.R.C. Unit of Insect Neurophysiology and Pharmacology, Department of Zoology, Cambridge University, Cambridge, U.K.; Laboratoire de Physiologie, Faculte de Medecine, Universite d'Angers, France; and the Department of Genetics, Albert Einstein College of Medicine, The Bronx, NY.

CHRONIC INHALATION STUDIES IN MICE. I. FACILITIES AND EQUIPMENT FOR "NOSE-ONLY" EXPOSURE TO CIGARETTE SMOKE

Facilities and equipment are described for large-scale, long-term "nose-only" inhalation exposure of mice to whole cigarette smoke. Experimental procedures and

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equipment were designed to provide the mice with exposure conditions where (1) the lung was the major target organ for the smoke, (2) large quantities of fresh, whole cigarette smoke could be generated, (3) large numbers of animals could be exposed at one time, (4) routine, daily exposures could be given over a major portion of the lifetime of the animal, (5) monitoring and documentation of the quantity of smoke presented to the animals was provided during each exposure session, (6) safety systems were provided that assured exposure of the animals to smoke only under pre-set exposure conditions, and (7) cigarette smoke was generated under conditions where factors, such as cigarette type, smoke aerosol concentration and smoke particle size, were controlled.

Henry, C. J. et al. (Microbiological Associates)

Beiträge zur Tabakforschung International 13(1):37-53, 1985.

From the Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, TN; Division of Toxicology and Molecular Biology, Microbiological Associates, Bethesda, MD; and Process and Instruments Corporation, Brooklyn, NY.

CALMODULIN-DEPENDENT NAD KINASE OF HUMAN NEUTROPHILS

NAD kinase from human neutrophils has been partially purified by sequential application of Red Agarose, ion-exchange, and gel-filtration chromatography. The enzyme has a broad pH optimum, 7.0-9.5, is strictly dependent upon the presence of Mg^{2+}, and in the absence of calcium exhibits K_m values of 0.6 and 0.9 mM for NAD and ATP, respectively. NAD kinase activity is extremely sensitive to free calcium concentration, with half-maximal activity observed at free calcium concentrations of approximately 0.4 μM. In cellular extracts, calcium-dependent activation of NAD kinase increases the maximum velocity of the reaction from 2- to 5-fold while not affecting K_m values for NAD and ATP. The activity of the partially purified NAD kinase is stimulated 3.5-fold by the addition of calmodulin in the presence of calcium. This stimulation is inhibited by the addition of 20 μM trifluoperazine to the incubation. These data are interpreted as implicating calmodulin in NAD kinase regulation. The total concentration of NADP + NADPH in the human neutrophil used increased 2.2-fold in response to activation by phorbol myristic acetate. Finally, neutrophil NAD kinase has a M_r, based upon gel filtration, of 169,000.

Williams, M. B. and *Jones, H. P.*

Biochemistry and Biophysics 237(1):80-87, 1985.

Other support: Kroc Foundation.

From the Department of Biochemistry, College of Medicine, University of South Alabama, Mobile.

A COMPARATIVE STUDY OF NEUTROPHIL PURIFICATION AND FUNCTION

Several different methods are currently used by numerous laboratories for the isolation of human neutrophils. These methods include partial purification by dextran sedimentation followed by water lysis, and more complete purification procedures utilizing discontinuous density gradients coupled with dextran sedimentation and in some cases hypotonic lysis. Some investigators refrain from using certain purification schemes because certain steps or reagents used in a particular method might adversely affect the

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functional parameter of the neutrophil they wish to measure. In spite of these concerns there has been no systematic comparison of the functional status of neutrophils prepared by the various methodologies. In this study we have compared 4 commonly used methods of isolation with neutrophil function. The results of this study indicate that while neutrophil yield and purity were determined by isolation procedure, all cells were equivalent with regard to chemotactic performance, ability to degranulate, and ability to produce superoxide and hypochlorous acid.

Grisham, M. W., Engerson, T. D., McCord, J. M., and *Jones, H. P.*

Journal of Immunological Methods 82:315-320, 1985.

Other support: The American Heart Association.

From the Department of Biochemistry, College of Medicine, University of South Alabama, Mobile.

ACTIVATION-ASSOCIATED ALTERATIONS IN NEUTROPHIL PYRIDINE NUCLEOTIDE LEVELS: A POTENTIAL REGULATORY ROLE FOR CALCIUM AND CALMODULIN

The concentration of NADP + NADPH in resting human neutrophils has been measured to be 24.0 ± 2.7 10^{-18}mol/cell. Upon activation with opsonized zymosan A, phorbol myristic acetate or *N*-formylmethionylleucylphenylalanine, neutrophil NADP + NADPH pools increase to 80.5, 84.0 and 54.0 × 10^{-18}mol/cell, respectively. These increases in pyridine nucleotide concentration are blocked by the addition of the calcium antagonist 8-(*N,N*-dimethylamino)-octyl-3,4,5-trimethoxybenzoate hydrochloride, while calcium ionophore A23187, in the presence of calcium, will trigger the increase in the absence of other stimuli. Calmodulin antagonists trifluoperazine and *N*-(6-aminohexyl)-5-chloro-1-naphthalene sulfonamide also inhibit stimulus-induced increases in the NADP + NADPH pool. These studies are interpreted as suggesting a role for calcium and calmodulin and possibly protein kinase C in the regulation of pyridine nucleotide concentration in the activated neutrophil.

Sparkman, T. B., Johns, T., Engerson, T., and *Jones, H. P.*

Biochimica et Biophysica Acta 846:8-13, 1985.

Other support: National Institutes of Health.

From the Department of Biochemistry, College of Medicine, University of South Alabama, Mobile.

EFFECT OF ALLOPURINOL ON NEUTROPHIL SUPEROXIDE PRODUCTION, [illegible] OR DEGRANULATION

Recent studies examining the effect of allopurinol on bacterial killing by leukocytes, have been interpreted by those authors as proof that xanthine oxidase is the major superoxide producing enzyme in activated leukocytes. To test the assertion that xanthine oxidase is involved in the production of superoxide by activated human neutrophils, the xanthine oxidase content of neutrophils was measured and the effect of allopurinol on neutrophil functions, including superoxide production, was studied. Neutrophils were found to contain a level of xanthine oxidase insufficient to account for the flux of superoxide associated with neutrophil activation. Allopurinol did not inhibit

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superoxide production induced by opsonized zymosan, phorbol myristic acetate or formyl-methionylleucylphenylalanine. Furthermore, neither chemotaxis nor degranulation was affected by allopurinol. Allopurinol was also found ineffective in blocking superoxide-mediated carrageenan-induced foot edema in the rat. These studies are interpreted as evidence that xanthine oxidase is not a major superoxide-generating system in activated neutrophils as has been suggested by others.

Jones, H. P., et al.

Biochemical Pharmacology 34(20):3673-3676, 1985.

Other support: National Institute of Arthritis, Metabolism, Digestive and Kidney Disease and the American Heart Association.

From the Department of Biochemistry, College of Medicine, University of South Alabama, Mobile.

INCREASED BETA-THROMBOGLOBULIN IN THE SERUM OF SMOKERS OCCURS BECAUSE OF INCREASED PLATELET COUNTS.

The amount of β-thromboglobulin (β-TG), a platelet alpha granule protein, released in the serum of smokers by thrombin formed during whole blood clotting was found to be significantly higher in smokers than in nonsmokers. This increase is most likely due to observed increased platelet numbers in smokers, rather than to increased platelet content of β-TG, since the concentration of β-TG per platelet is nearly identical in the two groups. An inverse relationship between release and platelet number was also found.

Longenecker, G.L., et al.

Research Communications in Substances of Abuse 5(2):147-152, 1984.

From the Department of Pharmacology, College of Medicine, University of South Alabama, Mobile.

MECHANISMS FOR REFLEXIVE HYPERTENSION INDUCED BY LOCAL APPLICATION OF CAPSAICIN AND NICOTINE TO THE NASAL MUCOSA

The cardiovascular effects of locally applied nicotine and capsaicin to the nasal mucosa were studied in anaesthetized guinea-pigs. Local application of capsaicin (0.3-30 μM) or nicotine (0.3-30 mM) induced dose-dependent increases in arterial blood pressure, mainly due to an increase in peripheral vascular resistance. The capsaicin and nicotine responses were abolished after local anaesthesia and markedly reduced (to about 20% of control) by combined pretreatment with phentolamine and propranolol, suggesting reflexogenic sympathetic activation. System capsaicin pretreatment abolished the hypertensive effect of capsaicin (30 μM) and reduced the response to nicotine application to about 25% of control ($p < 0.001$). Local capsaicin pretreatment of the nasal mucosa one week earlier also significantly reduced the capsaicin response ($p < 0.05$), while the nicotine-induced increase in blood pressure was not significantly changed. The present findings suggest the presence of two afferent mechanisms in the nasal mucosa which induce hypertension upon chemical irritation. The capsaicin response is dependent upon capsaicin-sensitive afferents. The nicotine response involves

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mainly capsaicin-sensitive neurons and, in addition, a minor component which is resistant to capsaicin pretreatment. Thus, the hypertensive effect of nicotine applied locally to the nasal mucosa seems to be mainly mediated via sensory mechanisms other than the sneezing response which is not dependent on capsaicin sensitive nerves.

Lundblad, L., Hua, X.-Y., and *Lundberg, J. M.*

Acta Physiologica Scandinavica 121:277-282, 1984.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, Astra Foundation, and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, and the Department of Oto-Rhino-Laryngology, Karolinska Hospital, Stockholm, Sweden.

EFFECTS OF NICOTINE ON BLASTOCYST DEVELOPMENT PRIOR TO IMPLANTATION IN THE RAT

The following study was undertaken to determine the effects of nicotine treatment on conceptus development during the initial 5 days of pregnancy. In addition, litters were monitored for long-term post-partum effects of nicotine exposure on growth and sexual maturation. To investigate possible modes of nicotine action, plasma progesterone levels were determined in treated (N) *vs* control (C) rats during the initial 5 days of pregnancy, and the effects of the alkaloid on uterine blood flow and intrauterine oxygen tension were measured in pseudopregnant rats. Litter size and birth weight were determined in C vs N rats and body growth subsequently plotted. The onset of puberty in females was determined by noting the day of vaginal opening. Results show that twice daily sc injection of nicotine during the first 5 days of pregnancy retards conceptus growth as indicated by the reduced number of cells per blastocyst. The same treatment had no effect on litter size, birth weight or body growth. However, the day of vaginal opening was delayed. Nicotine treatment generally suppressed plasma progesterone levels during the initial 5 days of pregnancy. Furthermore, a single injection of the alkaloid induced a rapid and sustained reduction in uterine blood flow and a concomitant decrease in intrauterine oxygen. As indicated by this work, nicotine may modify conceptus development by altering reproductive tract function. Nicotine-induced changes in serum progesterone levels and uterine blood flow probably result primarily from the alkaloid's diverse and complex actions on the nervous system. The present results confirm and extend previous investigations reporting effects of pre-implantation nicotine exposure on conceptus development and progesterone secretion. In addition, the present study demonstrates that even a relatively brief exposure to nicotine, *i.e.*, during the initial days of pregnancy is sufficient to modify the timing of puberty in the rat. The delay in onset of puberty suggests long-term nicotine induced modification of CNS function.

Mitchell, J. A. and Hammer, R. E.

In: Caciagli, F., Giacobini, E., and Paoletti, R. (eds.): *Developmental Neuroscience: Physiological, Pharmacological and Clinical Aspects*, New York: Elsevier Science Publishers B.V., 1984, pp. 151-155.

From the Department of Anatomy, Wayne State University School of Medicine, Detroit.

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

EFFECTS OF NICOTINE ON OVIDUCAL BLOOD FLOW AND EMBRYO DEVELOPMENT IN THE RAT

Nicotine (5.0 mg/kg) was injected (s.c.) twice daily on Day 1 or Days 1-4 or 1-5 of pregnancy. Cumulative doses of nicotine retarded embryo cell cleavage and substantially reduced embryo cell number (saline *vs.* nicotine: 42.5 ± 1.7 *vs* 22.1 ± 1.9 nuclei/embryo, at 12:00 h on Day 5; $P < 0.05$. However, treatment for even 1 day (Day 1) significantly reduced cell number (saline *vs.* nicotine: 42.5 ± 1.7 *vs.* 30.5 ± 0.9, at 12:00 h day on Day 5; $P < 0.01$). Nicotine injection also resulted in a marked and prolonged reduction in oviduct blood flow (pretreatment *vs* 90 min after nicotine: 0.61 ± 0.06 *vs.* 0.37 ± 0.10 ml $min^{-1} g^{-1}$; $P < 0.005$. The results indicate that, in the rat, even a brief exposure to nicotine, the chief alkaloid of tobacco, reduces oviducal blood flow and the rate of embryo cell proliferation. The embryo is therefore susceptible to the effects of nicotine before implantation.

Mitchell, J. A. and Hammer, R. E.

Journal of Reproduction & Fertility 74:71-76, 1985.

Other support: The Research Society of Sigma Xi.

From the Department of Anatomy, Wayne State University School of Medicine, Detroit.

SELECTIVE ACTIVATION OF PARTICULATE GUANYLATE CYCLASE BY A SPECIFIC CLASS OF PORPHYRINS

Guanylate cyclase was activated 3- to 10-fold by hemin in a dose-dependent manner in membranes prepared from homogenates of rat lung, C_6 rat glioma cells, or B103 rat neuroblastoma cells. Maximum activation was observed with 50 to 100 μM hemin with higher concentrations being inhibitory. Activation was observed when Mg^{2+}-GTP but not when Mn^{2+}-GTP was used as the substrate. Increased enzyme activity reflected selective activation of the particulate form of guanylate cyclase; hemin inhibited the soluble form of gyanylate cyclase 70 to 90% over a wide range of concentrations. Activation was not secondary to proteolysis since a variety of protease inhibitors failed to alter stimulation by hemin. Protophorphyrin IX had little effect on particulate guanylate cyclase activity and sodium borohydride almost completely abolished hemin-dependent activation. These data suggest a requirement for the ferric form of the porphyrin-metal chelate for activation. However, agents which interact with the iron nucleus of porphyrins, such as cyanide, had little effect on the ability of hemin to activate guanylate cyclase. The stimulatory effects of hemin were observed in the presence of detergents such as Lubrol-PX, and highly purified particulate enzyme could be activated to the same extent as enzyme in native membranes. These data suggest that the interaction of porphyrins with particulate guanylate cyclase is complex in nature and different from that with the soluble enzyme.

Waldman, S. A., Sinacore, M. S., Lewicki, J. A., Chang, L. Y. and *Murad, F.*

The Journal of Biological Chemistry 259(7):4038-4042, 1984.

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University, Stanford, CA and Veterans Administration Medical Center, Palo Alto, CA.

HIGHLY PURIFIED PARTICULATE GUANYLATE CYCLASE FROM RAT LUNG: CHARACTERIZATION AND COMPARISON WITH SOLUBLE GUANYLATE CYCLASE

Guanylate cyclase was purified 1000-fold from washed rat lung particulate fractions to a final specific activity of 500 nmoles cyclic GMP produced/min/mg protein by a combination of detergent extraction and chromatography on concanavalin A-Sepharose, GTP-agarose, and blue agarose. Particulate guanylate cyclase has a molecular weight of 200,000 daltons, a Stokes radius of 48Å and a sedimentation coefficient of 9.4, while the soluble form has a molecular weight of 150,000 daltons, a Stokes radius of 44Å, and a sedimentation coefficient of 7.0. Whereas the particulate enzyme is a glycoprotein with a specific affinity for concanavalin A and wheat germ agglutinin, the soluble form of guanylate cyclase did not bind to these lectins. Purified particulate guanylate cyclase did not cross-react with a number of monoclonal antibodies generated to the soluble enzyme. While both forms of the enzyme could be regulated by the formation of mixed disulfides, the particulate enzyme was relatively insensitive to inhibition by cystine. With GTP as substrate, both forms of the enzyme demonstrated typical kinetics, and with GTP analogues, negative cooperativity was observed with both enzyme forms. These data support the suggestion that the two forms of guanylate cyclase possess similar catalytic sites although their remaining structure is divergent, resulting in differences in subcellular distribution, physical characteristics and antigenicity.

Waldman, S. A., Lewicki, J. A., Chang, L. Y. and *Murad, F.*

Molecular and Cellular Biochemistry 57:155-166, 1983.

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University, Stanford, CA, and Veterans Administration Medical Center, Palo Alto, CA.

ENDOTHELIUM-DEPENDENT AND NITROVASODILATOR-INDUCED RELAXATION OF VASCULAR SMOOTH MUSCLE: ROLE OF CYCLIC GMP

The mechanisms by which endothelium-dependent relaxants and nitrovasodilators cause relaxation of vascular smooth muscle has been reviewed. A model explaining these observations is summarized in Fig. 1. The endothelium-dependent vasodilators through interaction with their appropriate receptors, are thought to activate phospholipase A_2 and cause the release of an unsaturated fatty acid. The released unsaturated fatty acid or a metabolite is thought to be the "endothelial relaxant factor" that interacts with the smooth muscle component to cause relaxation. While the unsaturated fatty acid may be oxidized in either the endothelial cell or smooth muscle cell, the lability of the endothelial relaxant factor suggests that at least some of this processing occurs before its release from the endothelium. The model in Figure 1 suggests that an oxidized fatty acid or a derived free radical is responsible for activation of smooth muscle guanylate cyclase and increases in cyclic GMP levels. As pointed out above, the use of various inhibitors of fatty acid

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release and metabolism has not allowed us or others to predict the structure of the active material. To date the best evidence suggests that the unsaturated fatty acid is a product of either the lipoxygenase or P-450 pathways.

Rapoport, R. M. and *Murad, F.*

Journal of Cyclic Nucleotide and Protein Phosphorylation Research 9(4-5):281-296, 1983.

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and Veterans Administration Medical Center, Palo Alto, CA.

ATRIAL NATRIURETIC FACTOR SELECTIVELY ACTIVATES PARTICULATE GUANYLATE CYCLASE AND ELEVATES CYCLIC GMP IN RAT TISSUES

A study was done on the effects on guanylate cyclase and cyclic GMP accumulation of a synthetic peptide containing the amino acid sequence and biological activity of atrial natriuretic factor (ANF). ANF activated particulate guanylate cyclase in a concentration- and time-dependent fashion in crude membranes obtained from homogenates of rat kidney. Activation of particulate guanylate cyclase by ANF was also observed in particulate fractions from homogenates of rat aorta, testes, intestine, lung, and liver, but not from heart or brain. Soluble guanylate cyclase obtained from these tissues was not activated by ANF. Trypsin treatment of ANF prevented the activation of guanylate cyclase, while heat treatment had no effect. Accumulation of cyclic GMP in kidney minces and aorta was stimulated by ANF activation of guanylate cyclase. These data suggest a role for particulate guanylate cyclase in the molecular mechanisms underlying the physiological effects of ANF such as vascular relaxation, natriuresis and diuresis.

Waldman, S. A., Rapoport, R. M. and *Murad, F.*

The Journal of Biological Chemistry 259(23):14332-14334, 1984.

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and Veterans Administration Medical Center, Palo Alto, CA.

ATRIAL NATRIUR[illegible]IC FACTOR ELICITS AN ENDOTHELIUM-INDEPENDENT RELAXATION AND ACTIVATES PARTICULATE GUANYLATE CYCLASE IN VASCULAR S[illegible] MUSCLE

A 26 amino acid synthetic peptide fragment of atrial natriuretic factor (ANF) relaxed isolated rabbit aortic segments in which the endothelium was either intact or functionally destroyed. The relaxations were temporally associated with increases in levels of cGMP with no change in the levels of cAMP. The ANF-induced increases in cGMP were also observed in aortic segments pretreated with calcium-free buffer or the cGMP phosphodiesterase inhibitor M&B-22,948. Qualitatively similar results were obtained for sodium nitroprusside. ANF selectively activated particulate guanylate cyclase, having no effect on the soluble form of the enzyme. Thus, the direct (endothelium-independent) vasodilator effect of ANF may be mediated via increased tissue levels of cGMP. ANF appears to increase vascular cGMP levels by activation of particulate guanylate cyclase.

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

Winquist, R. J., Faison, E. P., Waldman, S. A., Schwartz, K. *Murad, F.* and Rapoport, R. M.

Proceedings of the National Academy of Sciences of the United States of America 81: 7661-7664, 1984.

Other support: National Institutes of Health and Veterans Administration.

From the Department of Cardiovascular Pharmacology, Merck Institute for Therapeutic Research, West Point, PA; Departments of Medicine and Pharmacology, Stanford University, Stanford, CA; and Veterans Administration Medical Center, Palo Alto, CA.

MECHANISMS OF ADENOSINE TRIPHOSPHATE-, THROMBIN-, AND TRYPSIN-INDUCED RELAXATION OF RAT THORACIC AORTA

The mechanisms by which adenosine triphosphate, thrombin, and trypsin cause relaxation of vascular smooth muscle were investigated. Relaxation of the rat thoracic aorta with adenosine triphosphate, thrombin, and/or trypsin was associated with increased levels of cyclic guanosine monophosphate in both time- and concentration-dependent manners. Thrombin and trypsin did not alter cyclic adenosine monophosphate levels whereas adenosine triphosphate increased cyclic adenosine monophosphate levels after significant relaxation occurred. Removal of the endothelium abolished adenosine triphosphate-, thrombin-, and trypsin-induced relaxation and the associated increased levels of cyclic nucleotides. Relaxation due to these agents was also inhibited by exposure to nordihydroguaiaretic acid, a lipoxygenase inhibitor, and eicosatetraynoic acid, a lipoxygenase and cyclooxygenase inhibitor. Indomethacin, a cyclooxygenase inhibitor, potentiated relaxation to these agents, whereas the increased levels of cyclic nucleotides due to adenosine triphosphate were unaltered. Bromophenacyl bromide, a phospholipase A_2 inhibitor, decreased relaxation due to adenosine triphosphate, thrombin, and trypsin and the associated increased levels of cyclic nucleotides. Removal of extracellular calcium, which also presumably inhibits phospholipase A_2, prevented the elevated levels of cyclic nucleotides and the inhibitory effects of adenosine triphosphate and trypsin on contraction. In contrast, sodium nitroprusside-induced relaxation and/or increased levels of cyclic guanosine monophosphate were unaltered by nordihydroguaiaretic acid, eicosatetraynoic acid, bromophenacyl bromide, and removal of extracellular calcium. After incubation of intact tissue with ^{32}P-orthophosphate, the patterns of protein phosphorylation caused by adenosine triphosphate, thrombin, and trypsin were indistinguishable from those of acetylcholine, sodium nitroprusside and 8-bromo cyclic guanosine monophosphate. All these agents dephosphorylated myosin light chain. Thus, the present study supports the hypothesis that relaxation induced by adenosine triphosphate, thrombin and trypsin is mediated through the formation of an endothelial factor which elevates cyclic guanosine monophosphate levels and causes cyclic guanosine monophosphate-dependent protein phosphorylation and dephosphorylation of myosin light chain.

Rapoport, R. M., Draznin, M. B., and *Murad, F.*

Circulation Research 255/4:468-479, 1984.

Other support: National Institutes of Health, Veterans Administration, National Research Service Award.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and Veterans Administration Medical Center, Palo Alto, CA.

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ATRIOPEPTIN I ELEVATES CYCLIC GMP, ACTIVATES CYCLIC GMP-DEPENDENT PROTEIN KINASE AND CAUSES RELAXATION IN RAT THORACIC AORTA

Synthetic atriopeptin II, an atrial natriuretic factor with potent vasodilatory effects, was studied in isolated strips of rat thoracic aorta to determine its actions on contractility, cyclic nucleotide concentrations and endogenous activity of cyclic nucleotide-dependent protein kinases. Atriopeptin II was found to relax aortic strips precontracted with 0.3 μM norepinephrine whether or not the endothelial layer was present. Relaxation to atriopeptin II was closely correlated in a time- and concentration-dependent mannner with increases in cyclic GMP concentrations and activation of cyclic GMP-dependent protein kinase (cyclic GMP-kinase). The threshold concentration for all three effects was 1 nM. Atriopeptin II (10 nM for 10 min) produced an 80% relaxation, an 8-fold increase in cyclic GMP concentrations and a 2-fold increase in cyclic GMP-kinase activity ratios. Atriopeptin II did not significantly alter cyclic AMP concentrations or cyclic AMP-dependent protein kinase activity. These data suggest that cyclic GMP and cyclic GMP-kinase may mediate vascular relaxation to a new class of vasoactive agents, the atrial natriuretic factors. Similar effects have been observed with the nitrovasodilator, sodium nitroprusside, and the endothelium-dependent vasodilator, acetylcholine. Therefore, a common biochemical mechanism of action that includes cyclic GMP accumulation and activation of cyclic GMP-kinase may be involved in vascular relaxation to nitrovasodilators, endothelium-dependent vasodilators and atrial natriuretic factors.

Fiscus, R. R., Rapoport, R. M., Waldman, S. A., and *Murad, F.*

Biochimica et Biophysica Acta **846**:179-184, 1985.

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University, Stanford, CA; and Veterans Administration Medical Center, Palo Alto, CA.

A TWO-STEP PROCEDURE FOR OBTAINING HIGHLY PURIFIED PARTICULATE GUANYLATE CYCLASE FROM RAT LUNG

Guanylate cyclase (GTP pyrophosphate lyase (cyclizing), EC 4.6.1.2), the enzyme catalyzing the formation of cyclic GMP from GTP, exists in both soluble and membrane-bound forms. These enzymes have been implicated as key regulatory components in a variety of biological events such as secretion and smooth muscle relaxation. Understanding the role these enzymes play in cellular regulation is predicated upon obtaining purified preparations of guanylate cyclase. Several procedures for purifying the soluble enzyme to apparent homogeneity from a variety of tissues have been reported. The particulate enzyme has been purified to apparent homogeneity from sea urchin sperm but, to date, homogeneous preparations of particulate guanylate cyclase from mammalian tissues have not been obtained. Here we report our efforts in purifying particulate guanylate cyclase about 8,200- to 52,000-fold from homogenates of rat lung. Portions of this work have appeared in abstract form.

Waldman, S. A., Chang, L. Y., and *Murad, F.*

Preparative Biochemistry **15**(3):103-119, 1985.

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University, Stanford, CA, and Veterans Administration Medical Center, Palo Alto, CA.

EFFECTS OF ATRIOPEPTIN ON PARTICULATE GUANYLATE CYCLASE FROM RAT ADRENAL

Atriopeptin II activated particulate guanylate cyclase 5-10-fold in a concentration- and time-dependent fashion in crude membranes obtained from homogenates of rat adrenal cortex or medulla. Similar effects were observed with other atriopeptin analogs. Soluble guanylate cyclase and adenylate cyclase in these preparations were not activated. Accumulation of cyclic GMP in minces of adrenal cortex or medulla was increased 6-8-fold due to atriopeptin II activation of particulate guanylate cyclase. Several thiol-reactive agents blocked the activation of particulate guanylate cyclase, suggesting that free thiol groups on membrane proteins may be important in atriopeptin receptor-guanylate cyclase coupling.

Waldman, S. A., Rapoport, R. M., Fiscus, R. R., and *Murad, F.*

Biochimica et Biophysica Acta 845:298-303, 1985.

Other support: Veterans Administration.

From the Department of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and Veterans Administration Medical Center, Palo Alto, CA.

ROLE OF CYCLIC-GMP IN RELAXATIONS OF VASCULAR SMOOTH MUSCLE

Relaxation of rat aorta segments with sodium nitroprusside and endothelium-dependent vasodilators, such as acetylcholine, histamine, A23187, ATP, thrombin, and trypsin, is associated with cyclic-GMP (cGMP) accumulation in a concentration- and time-dependent fashion. With rat aorta segments, these agents also increase cyclic GMP-dependent protein-kinase activity and alter the incorporation of ^{32}P into numerous smooth-muscle proteins. Identical patterns of protein phosphorylation were observed with both classes of relaxants on two-dimensional gel electrophoresis and autoradiography. The effects of nitroprusside were observed with or without the endothelium present. In contrast, the effects of the endothelium-dependent agents on all of these parameters (cGMP, cGMP-dependent protein kinase and protein phosphorylation) required the integrity of the endothelium. Various inhibitors of phospholipase and lipoxygenase prevented the effects of the endothelium dependent agents, suggesting that a metabolite of arachidonic acid is the endothelium-relaxant factor and responsible for guanylate-cyclase activation. A smooth-muscle protein with decreased ^{32}P incorporation after treatment with either class of relaxants has been identified as myosin light chain. A model is presented suggesting that the effects of endothelium-dependent vasodilators and directly acting nitrovasodilators converge at the level of guanylate-cyclase activation and cGMP accumulation, which explains the common biochemical and physiological effects on smooth muscle of these two classes of vasodilators.

Murad, F., Rapoport, R. M. and Fiscus, R.

Journal of Cardiovascular Pharmacology 7 (Suppl. 3):S111-S118, 1985.

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and Veterans Administration Medical Center, Palo Alto, CA.

EFFECT OF SODIUM-POTASSIUM PUMP INHIBITORS AND MEMBRANE-DEPOLARIZING AGENTS ON SODIUM NITROPRUSSIDE-INDUCED RELAXATION AND CYCLIC GUANOSINE MONOPHOSPHATE ACCUMULATION IN RAT AORTA

The purpose of this study was to investigate the relationship between sodium nitroprusside-induced relaxation, inhibition of the sodium-potassium pump, and cyclic guanosine monophosphate. Exposure of rat thoracic aorta to ouabain, or potassium- or magnesium-free Krebs-Ringer bicarbonate solution, procedures which presumably inhibit the sodium-potassium pump, or to potassium chloride or tetraethylammonium, membrane-depolarizing agents, inhibited relaxation to nitroprusside. These conditions had little or no effect on the elevated cyclic guanosine monophosphate levels at a concentration of nitroprusside (0.1 μM) that relaxed norepinephrine contracted tissues by 80%. However, at a maximum relaxant concentration of nitroprusside (1.0 μM), these conditions decreased the elevation of cyclic guanosine monophosphate. The inhibition of elevated cyclic guanosine monophosphate levels was independent of the endothelium, extracellular calcium, and the cyclic guanosine monophosphate phosphodiesterase inhibitor, M&B 22,948. The inhibitory effects of ouabain and of potassium- and magnesium-free solution on the increased levels of cyclic guanosin monophosphate caused by 1.0 μM nitroprusside were abolished when tissues were incubated without norepinephrine, or with norepinephrine in the presence of the α-adrenergic blocker, phentolamine. In contrast, a β-adrenergic blocker, propranolol, had no effect on the ouabain-induced inhibition of elevated cyclic guanosine monophosphate levels, with norepinephrine present. These results are consistent with the hypothesis that membrane events regulate cyclic guanosine monophosphate synthesis. At nitroprusside concentrations greater than 0.1 μM, the formation of cyclic guanosine monophosphate appears to be coupled to the status of the smooth muscle cell membrane and the integrity of the sodium-potassium pump. Furthermore, the elevated cyclic guanosine monophosphate levels induced by nitroprusside may be compartmentalized and, depending upon the intracellular concentration, may be associated with several functions. These functions may include activation of the sodium-potassium pump and/or membrane hyperpolarization. However, an effect of cyclic guanosine monophosphate on sodium-potassium pump activity and/or membrane potential remains to be demonstrated.

Murad, F., Rapoport, R. M., and Schwartz, K.

Circulation Research 57(1):164-170, 1984.

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine and Veterans Administration Medical Center, Palo Alto, CA.

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

CALMODULIN-LANTHANIDE ION EXCHANGE KINETICS

A flow dialysis apparatus suitable for the study of high affinity metal binding proteins has been utilized to study calmodulin-metal exchange kinetics. Calmodulin labeled with Eu-155 and Gd-153 was dialyzed against buffer containing various competing metal ions. The rate of metal exchange was monitored by a gamma-ray scintillation detector. The kinetics of exchange are first order and the rates fall into two categories: Ca(II) and Cd(II) in one, and the lanthanides Eu(III), Gd(III), and LA(III) in the other.

Buccigross, J., O'Donnell, C., and *Nelson, D.*

Science Press, Beijing, China, pp. 406-409, 1985.

From the Department of Chemistry, Clark University, Worcester, MA.

A COMPARATIVE STUDY OF THE DISPOSITION OF NICOTINE AND ITS METABOLITES IN THREE INBRED STRAINS OF MICE

The disposition of nicotine, cotinine and nicotine N-oxide was investigated in male C57BL, DBA, and C3H mice following an ip injection of nicotine (1.0 mg/ml). The half-lives ($t_{\frac{1}{2}}$) of nicotine in blood were 5.9 to 6.9 min. The rapid elimination of nicotine was accompanied by a rapid accumulation of metabolites; maximal concentrations of cotinine in blood (204 to 364 ng/ml) were achieved in 10 min and nicotine N-oxide (23 ng/ml in C3H mice) in 15 min. The $t_{\frac{1}{2}}$ in blood was 20.1 to 39.8 min for cotinine and 18.4 min for nicotine N-oxide. The $t_{\frac{1}{2}}$ values for nicotine in brain were similar to those in blood, but the values for the liver were slightly larger (6.3 to 9.2 min) and interstrain differences were significant. A large strain-related difference in the $t_{\frac{1}{2}}$ for cotinine was found; the metabolite was eliminated from the blood of DBA mice at only about one-half the rate determined for the other strains. The $t_{\frac{1}{2}}$ for nicotine N-oxide in liver ranged from 12.7 to 27.3 min; the values were significantly different with C57BL > DBA and > C3H mice. Strain-related differences were also observed in response to chronic exposure to cigarette smoke. The $t_{\frac{1}{2}}$ of injected nicotine appeared to be slightly decreased in C57BL and DBA mice but was increased by 60% in livers of C3H mice compared to a control group.

Petersen, D. R., Norris, K. J. and Thompson, J. A.

Drug Metabolism and Disposition 12(6):G001-G007, 1984.

From the School of Pharmacy, University of Colorado, Boulder.

ISOLATION AND ANALYSIS OF N-OXIDE METABOLITES OF TERTIARY AMINES; QUANTITATION OF NICOTINE-1'N-OXIDE FORMATION IN MICE

To investigate the formation and elimination of nicotine-1'-N-oxide (NNO) in mice treated with a single injection of nicotine, sensitive and selective methods were developed to quantitate this polar and heat-labile metabolite. The compound was isolated from tissue homogenates as a dodecyl sulfate ion pair with C_{18} extraction cartridges and analyzed on an amino bonded-phase high-performance liquid chromatographic column with a mobile phase consisting of isopropanol-water. Overall recoveries of NNO were 64-76% from biological media. Several methods of detection were evaluated; radiolabeling was necessary to achieve the sensitivity required for

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

pharmacokinetic studies in mice. The *cis* and *trans* isomers of NNO were separated on a Partisil PAC column and enzymatic selectivity was evaluated for the formation of these isomers in mice.

Thompson, J. A., Norris, K. J. and *Petersen, D. R.*

Journal of Chromatography **341**:349-359, 1985.

From the School of Pharmacy, University of Colorado, Boulder.

INACTIVATION OF HUMAN α-1-PROTEINASE INHIBITOR BY GAS-PHASE CIGARETTE SMOKE

As reported in this study, direct exposure of human α1-proteinase inhibitor (α1PI) to the gas-phase smoke from one cigarette results in a biphasic inactivation of α1PI. (1) The authors observe a rapid, initial loss of elastase inhibitory capacity, the amount of which is dependent upon the age of the smoke. This rapid, *short-term inactivation* is not seen when the protein is exposed to aqueous extracts of cigarette smoke (as had been done in the past). (2) However, both exposure regimens produce a second, *slow inactivation* that occurs over several days. The authors suggest that the short-term inaction may be due to a peroxynitrate (or a similar reactive species) that is formed from radicals in the gas phase but is unstable in aqueous solution. The authors have not yet identified a species responsible for the long-term inactivation observed following both direct exposure to cigarette smoke and exposure to aqueous extracts. One possibility is that the inactivation comes about as a result of the cooxidation of α1PI with autoxidizable smoke compounds that dissolve in aqueous solutions.

Pryor, W. A., Dooley, M. M., and Church, D. F.

Biochemical and Biophysical Research Communications 122(2):676-681, 1984.

Other support: National Institutes of Health and the National Foundation for Cancer Research.

From the Departments of Chemistry and Biochemistry, Louisiana State University, Baton Rouge.

NITROSATION OF ORGANIC HYDROPEROXIDES BY NITROGEN DIOXIDE/DINITROGEN TETRAOXIDE

Cumyl and *tert*-butyl hydroperoxides react rapidly with NO_2 / N_2O_4 in organic solvents in the presence of a base to form the organic nitrate ($RONO_2$) as the major product, together with smaller amounts of the corresponding nitrite (RONO), alcohol, and carbonyl compound (acetophenone or acetone from cumyl and *tert*-butyl hydroperoxide, respectively). The products from *tert*-butyl hydroperoxide are similar whether a base is present or not but those from cumyl hydroperoxide are more complex. The researchers have formulated the initial reaction as a nitrosation of the hydroperoxide by N_2O_4 to give the peroxynitrite ester. This latter species is unstable and either rearranges to give the nitrate or dissociates to form alkoxyl radicals and nitrogen dioxide that ultimately gives the other observed products. The kinetics of the reaction were studied by stopped flow and are complex, but the investigators conclude they are

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consistent with the nitrosation mechanism. The rate constants at 30°C are 2.4×10^4 and 8.1×10^3 $M^{-1}s^{-1}$ for *tert*-butyl and cumyl hydroperoxides, respectively. The authors suggest that this facile reaction of NO_2 / N_2O_4 with hydroperoxides may have important consequences with respect to the pulmonary toxicity of NO_2 in smoggy air.

Pryor, W. A., Castle, L., and Church, D. L.

Journal of the American Chemical Society 107(1):211-217, 1985.

Other support: National Institutes of Health.

From the Departments of Chemistry and Biochemistry, Louisiana State University, Baton Rouge.

MECHANISMS OF CIGARETTE SMOKE TOXICITY: THE INACTIVATION OF HUMAN α-1-PROTEINASE INHIBITOR BY NITRIC OXIDE/ISOPRENE MIXTURES IN AIR

A mixture of nitric oxide (NO) and isoprene in air has been studied as a model for gas-phase cigarette smoke. We have shown that this model system duplicates many of the properties of cigarette smoke including the inactivation of human α-1-proteinase inhibitor (α1PI). In this study, buffered solution of α1PI were exposed to puffs of air containing 300 ppm NO and 400 ppm isoprene. Bubbling of the NO/air/isoprene gas stream directly through buffered protein solutions causes α1PI to undergo a fast loss of inhibitory capacity. This fast inactivation is not observed when α1PI is exposed to aqueous extracts of the NO/air/isoprene mixture. Both direct exposure and exposure to aqueous extracts, however, cause α1PI to undergo a slow loss of activity that continues for several days as the protein is incubated in the buffer solutions. Gas-phase cigarette smoke has already been show to cause this same two-phase inactivation of α1PI.

The inactivation of α1PI by the model system is dependent on the presence of oxygen in the gas stream, suggesting that the oxidation of nitric oxide to nitrogen dioxide in air is involved in the formation of the inactivating species. The nature of these species remains to be determined; however, small alkoxyl or peroxyl radicals (such as are spin-trappable from gas-phase smoke as well as from the NO/air/isoprene system) do *not* appear to inactivate α1PI. One possibility is that the inactivating species are metastable compounds formed by radical processes in the gas phase of both cigarette smoke and our model system. Our data suggest that one possible class of species is peroxynitrates.

Pryor, W. A., Dooley, M. M. and Church, D.F.

Biological Interactions 54:173-183, 1985.

Other support: National Institutes of Health and National Foundation for Cancer Research.

From the Departments of Chemistry and Biochemistry, Louisiana State University, Baton Rouge.

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INACTIVATION OF HUMAN ALPHA 1-PROTEINASE INHIBITOR BY CIGARETTE SMOKE: EFFECT OF SMOKE PHASE AND BUFFER

In order to resolve a discrepancy in the literature, we have examined the *in vitro* inactivation of human α1-proteinase inhibitor by direct exposures either to whole cigarette smoke or to filtered (*i.e.*, gas-phase) smoke. Wyss and coworkers reported that whole smoke does not inactivate the protein, whereas we reported that gas-phase smoke does. We now find that direct exposure to gas-phase cigarette smoke causes a slightly greater inactivation of the protein than does direct exposure to whole cigarette smoke, confirming our earlier suggestion that whole smoke is less oxidizing than is gas-phase smoke. This difference, however, does not explain the dramatic difference between our previous findings and those of Wyss and coworkers. The explanation for the discrepancy lies in the nature of the buffers used. Wyss and coworkers used Tris buffer and the use of Tris quenches the activation process almost completely. Our experiments used phosphate buffer. We suggest that Tris is an unsuitable buffer for use in experiments that probe the effects of cigarette smoke.

Pryor, W. A. and Dooley, M. M.

American Review of Respiratory Disease 131:941-943, 1985.

Other support: National Institutes of Health and National Foundation for Cancer Research.

From the Departments of Chemistry and Biochemistry, Louisiana State University, Baton Rouge.

FREE-RADICAL CHEMISTRY OF CIGARETTE SMOKE AND ITS TOXICOLOGICAL IMPLICATIONS

Cigarette smoke contains two very different populations of free radicals, one in the tar and one in the gas phase. The tar phase contains several relatively stable free radicals; we have identified the principal radical as a quinone/hydroquinone (Q/QH_2) complex held in the tarry matrix. We suggest that this Q/QH_2 polymer is an active redox system that is capable of reducing molecular oxygen to produce superoxide, eventually leading to hydrogen peroxide and hydroxyl radicals. In addition, we have shown that the principal radical in tar reacts with DNA *in vitro*, possibly by covalent binding.

The gas phase of cigarette smoke contains small oxygen- and carbon-centered radicals that are much more reactive than are the tar-phase radicals. These gas-phase radicals do not arise in the flame, but rather are produced in a steady state by the oxidation of NO to NO_2, which then reacts with reactive species in smoke such as isoprene. We suggest that these radicals and the metastable products derived from these radical reactions may be responsible for the inactivation of α_1-proteinase inhibitor by fresh smoke.

Cigarette smoke oxidizes thiols to disulfides; we suggest the active oxidants are NO and NO_2. The effects of smoke on lipid peroxidation are complex, and this is discussed. We also discuss the toxicological implications for the radicals in smoke in terms of a number of radical-mediated disease processes, including emphysema and cancer.

Church, D. F. and *Pryor, W. A.*

Environmental Health Perspectives 64:111-126, 1985.

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Source: https://www.industrydocuments.ucsf.edu/docs/sqxk0000

Other support: National Institutes of Health and the National Foundation for Cancer Research.

From the Departments of Chemistry and Biochemistry, Louisiana State University, Baton Rouge.

UTERINE BLOOD FLOW AND CATACHOLAMINE RESPONSE TO REPETITIVE NICOTINE EXPOSURE IN THE PREGNANT EWE

In order to simulate human smoking, experiments were designed to determine what dose of nicotine in the pregnant sheep would produce those plasma nicotine concentrations observed in human smokers and to measure uterine blood flow and plasma catecholamines in response to repetitive exposure (every 30 minutes) to that nicotine dose. Utilizing seven chronically catheterized pregnant sheep equipped with electromagnetic flow probes around both uterine arteries, we observed that a nicotine dose of 0.2 mg/min for 5 minutes results in mean plasma nicotine concentrations of 23.1 ± 1.1 ng/ml SEM (n = 17) immediately following infusion. This dose of nicotine was then infused every 30 minutes for 4 hours, and aliquots of blood were drawn immediately before and after nicotine infusion for determination of plasma catecholamines. No significant alterations in plasma epinephrine and norepinephrine were observed throughout the experiments (n = 8), and no significant changes in uterine blood flow occurred at any time during the experiment (n = 30). We conclude that there is a species difference between sheep and man with respect to the nicotinic threshold for catecholamine release.

Resnik, R., Conover, W. B., Key, T. C., and Van Vunakis, H.

American Journal of Obstetrics 151(7):885-889, 1985.

Other support: U. S. Public Health Service

From the Department of Reproductive Medicine, University of California at San Diego, School of Medicine, and the Department of Biochemistry, Brandeis University, Waltham, MA.

TREATMENT OF DIALYSIS MEMBRANES FOR SIMULTANEOUS DIALYSIS AND CONCENTRATION.

Dialysis membranes used for simultaneous dialysis-concentration required pretreatment to remove uv-absorbing compounds leached from the membranes and to reduce the absorption of protein to the membranes. This was accomplished with sodium carbonate and ethanol or with "sulfur-removal solutions." Protein determinations were made with a micro-Bradford protein reaction and with uv absorbance at 280 nm. Soluble membrane components contributed to [illegible] uv spectra and altered the ratio of 280/260-nm absorbance. Simultaneous dialysis and concentration in the micro protein dialyzer-concentrator apparatus, combining aspects of thin-layer dialysis and ultrafiltration, resulted in rapid removal of salts from the protein solutions. Prior treatment of membranes reduced uncertainties in retentate recoveries, eliminated uv-absorbing components of membranes, and improved recoveries of protein.

Richmond, V. L., St. Denis, R., and Cohen, E.

Analytical Biochemistry 145:343-350, 1985.

From the Pacific Northwest Research Foundation, Seattle, WA.

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STRUCTURAL REQUIREMENTS FOR ALLOSTERIC ACTIVATORS OF RAT LIVER MICROSOMAL 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE

Several compounds containing various structural moieties of NAD(P)(H), were examined as possible effectors of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Microsomal reductase was activated with 4.5 mM GSH, assayed with subsaturating NADPH concentration and increasing amounts of the tested compounds. Under these conditions, the essential and sufficient structure required to allosterically enhance the activity of the reductase is that of 5'-AMP. When the 2' position of the nucleotide is phosphorylated, this allosteric activation is diminished.

Roitelman, J. and *Shechter, I.*

Biochemical and Biophysical Research Communications 125(3):902-907, 1984.

From the Department of Biochemistry, the George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel.

ALLOSTERIC ACTIVATION OF RAT LIVER MICROSOMAL 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE BY NICOTINAMIDE ADENINE DINUCLEOTIDES

NADH and NAD^+ are neither substrates nor inhibitors of 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA) reductase in concentrations up to 1 mM. Addition of either NADH or NAD^+ enhanced the activity of rat liver microsomal reductase, yet NADH failed to affect the activity of the freeze-thaw solubilized enzyme. The degree of enhancement of enzyme activity by NADH decreased as GSH concentration in the assay increased. Addition of 500 μM NADH to the assay converted the sigmoidal (Hill coefficient = 2.0) NADPH-dependent kinetic curve of the microsomal reductase into Michaelis-Menten kinetics (Hill coefficient = 1.1). Furthermore, the kinetic curves were shifted to the left, resulting in an up to 35% decrease in the concentration of NADH required to obtain half-maximal velocity ($S_{0.5}$) in the presence of 500 μM NADH. Again, this effect of NADH was diminished as GSH concentrations increased. These results demonstrate that NAD(H) is an allosteric activator of HMG-CoA reductase. These results also indicate that HMG-CoA reductase has NAD(H) binding site(s) distinct from the catalytic NADPH site(s).

Roitelman, J. and *Shechter, I.*

The Journal of Biological Chemistry 259(22):14029-14032, 1984.

From the Department of Biochemistry, the George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel.

REDUCED GLUTATHIONE IN CHINESE HAMSTER OVARY CELLS PROTECTS AGAINST INACTIVATION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE BY 2-MERCAPTOETHANOL DISULFIDE

When the disulfide of 2-mercaptoethanol (ESSE) is added to the medium of cultured Chinese hamster ovary (CHO) cells, a time and concentration dependent release of 2-mercaptoethanol to the medium is observed. The reduction of ESSE to 2-mercaptoethanol by cells is a saturable process, the rate being approximately 50 nmoles

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of 2-mercaptoethanol per mg cell protein for an hour upon exposure to 250 μM ESSE. Reduction rate of ESSE by cells attached to a substratum is independent of glucose and insulin for periods up to 4 hours. However, in detached cells, swirled in suspension, addition of glucose and insulin is necessary in order to obtain a linear reduction rate of ESSE. The rate limiting enzyme in the sterol biosynthetic pathway, 3-hydroxy-3-methylglutaryl Coenzyme A reductase (E.C. 1.1.1.34), is inhibited by ESSE when isolated from CHO cells but total nonsaponifiable lipids synthesis from [2-^{14}C]-acetate in intact cells is not affected by ESSE at concentrations up to 500 μM. Cytosolic reduced glutathinone can spontaneously exchange disulfide bonds with ESSE and thus prevent it from inhibiting the reductase. Cultured cells respond to ESSE administration by elevating their total and acid-soluble glutathione levels. The use of ESSE as a pertubant of the GSH Status in cells is discussed.

Dotan, I. and *Shechter, I.*

Journal of Cellular Physiology 122:14-20, 1985.

From the Department of Biochemistry, the George S. Wise Faculty for Life Sciences, Tel Aviv University, Israel.

ADENOSINE-INDUCED CORONARY RELEASE OF PROSTACYCLIN AT NORMAL AND LOW pH IN ISOLATED HEART OF RABBIT

1. Rabbit hearts were perfused by the Langendorff method with drug-free perfusion medium or with a medium containing adenosine (10^{-7}M - 10^{-3}M) and the coronary and transmyocardial efflux rates of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) were measured. Perfusion was performed both at pH 7.4 and 6.9.

2. In other experiments the hearts were pre-labeled with [^{14}C]-arachidonic acid and the coronary efflux of radioactivity and of labeled lipids and 6-keto-$PGF_{1\alpha}$ was determined.

3. The basal coronary flow was elevated by almost 70% during tissue acidosis, in comparison to control. Adenosine induced a dose-dependent increase in the coronary flow, amounting to about 75% at normal pH and a drug concentration of 10^{-5}M. The adenosine-induced increase in coronary flow was not facilitated by low pH.

4. The base coronary efflux of 6-keto-$PGF_{1\alpha}$ from the hearts was 2.5-3.6 ng/min. Adenosine (10^{-6}-10^{-5}M) significantly facilitated this efflux, up to 6.5 ng/min. The efflux of 6-keto-$PGF_{1\alpha}$ was not changed by perfusion with acidic medium, either in the basal state or during perfusion with adenosine.

5. The basal interstitial efflux of 6-keto-$PGF_{1\alpha}$ was 4.5-5.5 ng/min. This efflux was not affected by perfusion of the heart with adenosine-containing medium. In hearts pre-labeled with [^{14}C]-arachidonic acid, adenosine (10 μM) induced a specific liberation of labeled lipid-extractable substances, including 6-keto-$PGF_{1\alpha}$.

From these data we conclude that adenosine stimulates the liberation of 6-keto-$PGF_{1\alpha}$ from the rabbit heart by increasing precursor availability and subsequent formation of prostacyclin in the coronary vessels. Furthermore, the increase in coronary flow induced by tissue acidosis is not related to an augmented formation of prostacyclin.

Ciabattoni, G. and *Wennmalm, Å.*

British Journal of Pharmacology 85:557-563, 1985.

Other support: Swedish Medical Research Council.

From the Department of Pharmacology, Catholic University, Rome, Italy, and the Department of Clinical Physiology at Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden.

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CHARACTERIZATION OF PROTEIN KINASE C ACTIVITY IN INTERFERON GAMMA TREATED MURINE PERITONEAL MACROPHAGES

Macrophage activation for tumoricidal microbicidal functions can be achieved in part by treatment with recombinant interferon gamma (IFNγ) *in vitro*. We have previously demonstrated that IFNγ treatment of murine peritoneal macrophages results in a two- to five-fold increase in the activity of Ca^{++} phospholipid dependent protein kinase C. We now report that this effect was not dependent upon continuing protein synthesis, since treatment with cycloheximide under conditions where normal protein synthesis was inhibited by greater than 95% had no effect upon the development of increased enzyme activity. Examination of Ca^{++} and phospholipid requirements revealed no differences between enzyme isolated from control or IFNγ-treated cells could not be distinguished in terms of the diacyglycerol (DC) or phorbol diester (PMA) concentration required for stimulation of activity. Kinetic analysis of the ATP (as substrate) concentration dependence revealed that both control and treated enzyme preparations (either basal or stimulated) had comparable Km values. Maximum velocity (Vmax) was increased both by IFNγ treatment and also by stimulation with DG or PMA. The major difference which could be discerned between protein kinase C derived from control versus IFNγ-treated macrophages was the magnitude of the response to DG or PMA; IFNγ treatment increased the stimulation index (*i.e.*, ratio of basal to stimulated activity) by a factor of two- to four-fold. These results suggest that IFNγ treatment leads to reversible modulation of existing protein kinase C resulting in increased catalytic activity when exposed to an appropriate stimulant.

Becton, D.L., *Adams. D.O.*, and Hamilton, T.A.

Journal of Cellular Physiology 125:485-491, 1985.

Other support: United States Public Health Service.

From the Departments of Pediatrics, Pathology and Microbiology-Immunology, Duke University, Durham, NC.

IDENTIFICATION OF THE LYMPHOKINE SOLUBLE IMMUNE RESPONSE SUPPRESSOR IN URINE OF NEPHROTIC CHILDREN

Patients with minimal change nephrotic syndrome (MCNS) frequently have suppressed *in vivo* and *in vitro* immune responsiveness of uncertain etiology. Because increased suppressor cell activity has been associated with this disease, urines from MCNS patients were screened for activity of the lymphokine soluble immune response suppressor (SIRS), a product of concanavalin A- or interferon-activated suppressor T cells. Urines from untreated MCNS patients suppressed polyclonal plaque-forming cell responses of cultured splenocytes. This suppressive activity was identified as human SIRS by the following functional and physical criteria: (a) molecular weight estimated by gel filtration; (b) kinetics of suppression; (c) inhibition of suppression by catalase, levamisole, and 2-mercaptoethanol; (d) abrogation of activity by acid or protease treatment; (e) elution pattern on high performance liquid chromatography; and (f) cross-reactivity with monoclonal antimurine SIRS antibodies. Suppressive activity disappeared from urine after initiation of treatment but before remission of symptoms.

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Urines were tested from 11 patients with MCNS, all of whom excreted SIRS. In addition, two nephrotic patients with acute glomerulonephritis and three nephrotic patients with membranoproliferative disease excreted SIRS, but other nephrotics and all nonnephrotic patients did not. These results indicate that excretion of SIRS occurs in certain cases of nephrotic syndrome and that the presence of SIRS in the urine is not accounted for solely by the presence of proteinuria or nephrosis. Serum from four nephrotic patients also contained SIRS, whereas neither serum nor urine from six normal subjects had SIRS activity. The systemic presence of SIRS in these four patients and the identification of SIRS in urines from a larger group of patients suggest a possible role for SIRS in the suppressed immune responses often found in nephrotic syndrome.

Schnaper, H.W. and *Aune, T.M.*

Journal of Clinical Investigation 76:341-349, 1985.

Other support: Monsanto Company.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis and the Departments of Pediatrics and Pathology, Washington University School of Medicine, St. Louis.

INHIBITION OF SOLUBLE IMMUNE RESPONSE SUPPRESSOR ACTIVITY BY GROWTH FACTORS

Soluble immune response suppressor (SIRS), a protein of M_r 14,000, is a lymphokine produced by interferon- or concanavalin A-activated suppressor T cells and is oxidized to its activated form, $SIRS_{ox}$, by hydrogen peroxide produced by macrophages. $SIRS_{ox}$ inhibits antibody secretion by B lymphocytes and cell division by normal or transformed cell lines. Effects of purified growth factors on suppression of antibody secretion were examined to determine whether any would oppose the inhibitory effects of SIRS or $SIRS_{ox}$. Interleukin 1 (IL-1), interleukin 2 (IL-2) and epidermal growth factor (EGF) each inhibited SIRS-mediated suppression of antibody secretion by cultured mouse spleen cells. Inhibition of SIRS activity was most effective when growth factors were added late in the culture period. IL-1, IL-2 and EGF also blocked suppression by $SIRS_{ox}$. However, EGF and IL-1 blocked suppression by $SIRS_{ox}$ only when added 3-6 hr before addition of $SIRS_{ox}$, whereas IL-2 blocked suppression by $SIRS_{ox}$ when added before or up to 3 hr after addition of $SIRS_{ox}$. Further evaluation showed that IL-2, but not IGF or IL-1, reversed inhibition of antibody secretion by $SIRS_{ox}$ in a time- and concentration-dependent manner. With 50 units of IL-2 per 0.5-ml culture, reversal was complete within 1 hr. The ability of growth factors to interfere with inhibition of cell division by $SIRS_{ox}$ was examined with the human B-cell leukemia RPMI-1788. This cell line binds EGF but is not known to have cell surface receptors for IL-1 or IL-2. EGF (0.3-1 ng/ml), when added to RPMI-1788 cultures 4-6 hr before $SIRS_{ox}$, interfered with the ability of $SIRS_{ox}$ to inhibit cell division. Taken together, these data indicate that growth factors interfere with both the immunosuppressive and growth inhibitory properties of $SIRS_{ox}$ in both heterogeneous and homogeneous cell populations.

Aune, T.M.

Proceedings of the National Academy of Sciences of the United States of America 82:6260-6264, 1985.

Other support: Science Foundation and the Monsanto Company.

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From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis; and the Department of Pathology, Washington University School of Medicine, St. Louis.

PRODUCTION OF THE LYMPHOKINE SOLUBLE IMMUNE RESPONSE SUPPRESSOR (SIRS) DURING CHRONIC EXPERIMENTAL SCHISTOSOMIASIS MANSONI

Chronic schistosomiasis mansoni is a helminthic infection characterized by cell-mediated, anti-egg granulomatous reactions and a variety of associated immunoregulatory phenomena. Soluble immune response suppressor (SIRS) is a lymphokine produced by activated suppressor T lymphocytes in various experimental settings. This report demonstrates the presence of SIRS in the sera of mice with chronic schistosomiasis mansoni (at least 20 wk of infection), but not in the sera of mice with earlier infections. Also, cultures of isolated, intact, hepatic, egg-focused granulomas from chronically infected mice release detectable levels of SIRS. These are the immunomodulated lesions characteristic of this infections. Large, intense, unmodulated granulomas obtained from acutely infected mice did not release SIRS. There is, therefore, a strong association between the presence of SIRS in the serum, the production of SIRS by intact lesions, and the chronic, immunomodulated stage of schistosomiasis mansoni.

Aune, T.M., Freedman, G.L. Jr., and Colley, D.G.

The Journal of Immunology 135(4):2768-2771, 1985.

Other support: National Science Foundation, National Institutes of Health and Veterans Administration.

From the Department of Pathology and Laboratory Medicine, the Jewish Hospital of St. Louis; the Department of Pathology, Washington University School of Medicine, St. Louis; Veterans Administration Medical Center and Department of Microbiology, Vanderbilt University School of Medicine, Nashville, TN.

TWO DIFFERENT PATHWAYS OF INTERFERON MEDIATED SUPPRESSION OF ANTIBODY SECRETION

Interferon suppresses a variety of *in vitro* immune responses by a mechanism which has not been well defined. Both direct suppression and activation of suppressor T cells have been suggested as possible mechanisms of interferon action. In an attempt to examine this question, interferon-α (IFNα)-mediated suppression of a plaque forming cell response to a T cell independent antigen by spleen cells or by B cells was examined. Somewhat greater quantities of IFNα were required to suppress plaque forming cell responses by B cells than by spleen cells to the antigen fluoresceinated-*Brucella abortus* (FITC-BA). However, suppression of spleen cell responses could be blocked by addition of either 2-mercaptoethanol, levamisole or monoclonal antibodies against the lymphokine, soluble immune response suppressor (SIRS), whereas suppression of B cell responses by IFNα was unaffected by these agents. Each of these agents interferes with SIRS mediated suppression of immune responses. Addition of T cells to B cell cultures stimulated with FITC-BA did not affect the total plaque forming cell response nor the extent of suppression by IFNα, but it did restore 2-mercaptoethanol sensitivity to IFNα-mediated suppression. As few as 1×10^5 T cells were effective and it was necessary to add T cells within 3 h of addition of IFNα to confer 2-mercaptoethanol

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sensitivity to IFNα-mediated suppression. These data suggest that IFNα can suppress immune responses by two different pathways and that in the presence of T cells, activation of suppressor T cells is the dominant pathway. The presence of T cells must also prevent direct suppression of B cells by IFNα.

Aune, T.M.

International Journal of Immunopharmacology 7(1):65-71, 1985.

Other support: Monsanto Company.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, and the Department of Pathology, Washington University School of Medicine, St. Louis.

INHIBITION OF NATURAL KILLING BY ADENOSINE RIBONUCLEOTIDES

The effect of nucleotides on natural killer activity in mouse spleen was investigated. Adenosine ribonucleotides were found to strongly inhibit natural killer activity, with a half maximal inhibition at an ATP-concentration of 2.0 μM. The inhibition was highly specific for adenosine ribonucleotides; adenosine deoxyribonucleotides, guanosine deoxyribonucleotides, and guanosine ribonucleotides had very low, if any, inhibitory effect. Experiments with specific inhibitors showed that the effect is transmitted by purinergic receptor(s) located on the cell surface. Comparison of the kinetics of the ATP effect with the effects of trifluoperazine and quinacrine showed that ATP acts late in the lytic cycle, possibly immediately preceding the programming for lysis.

Henriksson, T. *(Blair, P.B.)*

Immunology Letters 7:171-176, 1983.

Other support: U. S. Public Health Service and the National Cancer Institute.

From the Department of Microbiology and Immunology, and the Cancer Research Laboratory, University of California, Berkeley.

ANTI-MY-26: A MONOCLONAL ANTIBODY INHIBITING HUMAN PHAGOCYTE CHEMILUMINESCENCE

Anti-My-26, a mouse monoclonal IgG1 antibody, was raised against human granulocytes and has been shown to inhibit luminol-enhanced, glucose-independent chemiluminescence (CL) of human granulocytes (or monocytes) responding to the soluble secretagogues A23187 or ionomycin (calcium ionophores) and phorbol myristate acetate (PMA). Anti-My-26 inhibition of CL was reversible and was dependent on both secretatogue and monoclonal antibody concentration. This inhibition appeared to be directed at the component of granulocyte CL that is independent of NAD(P)H-oxidase-catalyzed formation of superoxide anion, because neither opsonized zymosan-stimulated CL nor the PMA-induced decrease in NAD (P)H-associated autofluorescence was affected by anti-My-26. In addition, ionomycin, over a wide concentration range, failed to generate any decrease in granulocyte autofluorescence. The A32187-induced CL inhibited by anti-My-26 was correlated with its depression of oxygen consumption. Furthermore, anti-My-26 was not cytotoxic and did not itself

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induce oxidative metabolism when used as a stimulant. Binding of anti-My-26 to phagocytic cells was not decreased by pre-exposure of cells to either A23187 or PMA. Evidence is presented to suggest that the binding of anti-My-26 to the granulocyte surface inhibits the oxidative response to calcium ionophore and PMA by blocking a common pathway(s) stimulated by these different secretagogues.

Warren, L. T. and *Civin, C.I.*

The Journal of Immunology 134(3):1827-1835, 1985.

Other support: National Institutes of Health.

From the Department of Biology, University of Pennsylvania, Philadelphia, and the Division of Pediatric Oncology, The Johns Hopkins Oncology Center, The Johns Hopkins University School of Medicine, Baltimore.

ANTI-MY-28, AN ANTIGRANULOCYTE MOUSE MONOCLONAL ANTIBODY, BINDS TO A SUGAR SEQUENCE IN LACTO-N-NEOTETRAOSE

Anti-My-28 is an IgM K monoclonal antibody produced by a hybridoma prepared from spleen cells of a mouse immunized with normal human granulocytes. By immunofluorescence it binds to human granulocytes but not to monocytes and lymphocytes. However, after treating cells with neuraminidase, the antibody also binds to lymphocytes and monocytes and to many leukemic cell lines and patient leukemic blast cells. Anti-My-28 binds to several neutral glycolipids and desialylated gangliosides of leukocytes and erythrocytes as shown by radioimmunoassay and immunostaining of thin-layer chromatograms. It recognizes a sugar sequence in lacto-N-neotetraose, Galβ1-4 GlcNAcβ1-3Galβ-4Glc. This tetrasaccharide occurs in the glycolipids paragloboside and sialosylparagloboside, and its distal trisaccharide sequence is found in higher glycolipids and glycoproteins.

Spitalnik, S. L., *Civin, C. I. et al.*

Blood 66(2):319-326, 1985.

Other support: National Institutes of Health.

From the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Bethesda, MD, and the Division of Pediatric Oncology, the Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, MD.

MIXED GLYCOSIDE PRETREATMENT REDUCES NONSPECIFIC BINDING OF ANTIBODIES TO FROZEN TISSUE SECTIONS

Indirect immunohistochemical studies of frozen mouse tissues with mouse monoclonal antibodies yield, in general, suboptimal results primarily because of indiscriminate binding of secondary antibody to all mouse immunoglobulins, i.e., to the monoclonal reagent and to endogenous immunoglobulin nonspecifically trapped in the tissue. To reduce this nonspecific staining, frozen sections of mouse kidney were treated enzymatically. Optimal results were obtained following a 2 hr treatment with 20 mg/ml of mixed glycosidases (MG). This treatment reduced the nonspecific background staining of the interstitial spaces and blood vessels, but did not affect the reactivity of structurally bound immunoglobulin G (IgG) in the glomeruli or alter the reactivity of

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mouse renal tissue to the monoclonal antibody that recognizes an oligosaccharide antigenic determinant. Eluates from enzyme-treated frozen tissue sections contained normally immunoreactive IgG in the form of dimers. These data indicate that MG treatment of frozen sections could be safely used to reduce the content of nonstructurally bound immunoglobulins in frozen tissues and thus improve the visualization of specific monoclonal antibody binding.

Andrews, L. P., Clark, R. K., and *Damjanov, I.*

The Journal of Histochemistry and Cytochemistry **33**(7):695-698, 1985.

Other support: National Institutes of Health.

From the Department of Pathology and Laboratory Medicine, Hahnemann University School of Medicine, Philadelphia.

LOCALIZATION OF cAMP IN DOG AND CAT TRACHEA: EFFECTS OF β-ADRENERGIC AGONISTS

Adenosine 3',5'-cyclic monophosphate (cAMP) is believed to mediate the transport of ions, water, and mucous glycoproteins in the respiratory tract. Because chemical measurements of total tissue levels of cAMP may not always reflect changes in specific cell types, we adapted standard immunocytochemical methods to examine the cellular localization of cAMP in dog and cat tracheae. The β-adrenergic agonists terbutaline and isoproterenol increased immunoreactive cAMP in ciliated epithelial cells of dog and cat tracheae and in both serous and mucous gland cells of cat tracheae. Epithelial goblet cells did not respond to β-adrenergic agonists in either species. This study provides information about the location of β-receptors on individual cells in the trachea that is not available from chemical assays of either cAMP or β-receptors in these tissues. Our results support the hypothesis that secretory functions in both serous and mucous submucosal gland cells and ciliated epithelial cells, but not goblet cells, may involve cyclic AMP-dependent mechanisms.

Lazarus, S. C., Basbaum, C. B. and *Gold, W. M.*

American Journal of Physiology **247** (Cell Physiology 16):C327-C334, 1984.

Other support: National Heart, Lung and Blood Institute, and the Strobel Medical Research Fund of the American Lung Association of San Francisco.

From the Cardiovascular Research Institute and Departments of Medicine and Anatomy, University of California, San Francisco.

IMMUNOLOGIC RELEASE OF HISTAMINE FROM DOG LUNG: COMPARISON OF IN VIVO AND IN VITRO RESPONSES IN THE SAME ANIMAL

The purpose of this study was to compare, for the first time, antigen-induced histamine release from the lung in the same natively allergic dogs both *in vitro* and *in vivo*. In six dogs, maximal antigen-induced histamine release from the lung correlated closely *in vitro* and *in vivo* (r=0.94), although it varied widely between dogs (0% to 75.5% of total tissue histamine content); similarly, the antigen concentration to produce 50% of maximal histamine release varied sixfold between dogs (40 μg/ml to 250 μg/ml). In each

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of five other dogs, terbutaline sulfate administered intravenously caused a dose-dependent inhibition of antigen-induced histamine release from lung fragments *in vitro*: the maximal inhibition produced by 1 mg/kg was 60 ± 4.5% (mean ± SEM). In these same dogs, 10^{-6}M terbutaline incubated with lung fragments *in vitro* caused inhibition of antigen-induced histamine release comparable to 1 mg/kg terbutaline *in vivo*. Increasing the dose of terbutaline *in vitro* produced maximal inhibition at 10^{-4}M with no greater effect of the drug at 10^{-3}M (71.4±3.8% inhibition). In both experimental situations propranolol caused a dose-dependent inhibition of β-adrenergic modulation of *Ascaris*-induced release of histamine. This result supports the conclusion that terbutaline produced its effects by actions mediated by β-adrenergic receptors on pulmonary mast cells. This experimental approach provides a suitable preparation in which to estimate the effective dose of agonists that modulate antigen-induced mast cell function *in vivo*.

Frey, M. J., Chesrown, S. E., Reed, B. R., Greenspon, L. W., Shields, R. L., Bourne, H. R., Brown, J. K., and *Gold, W. M.*

Clinical Immunology 74(5):728-729, 1984.

Other support: U. S. Public Health Service Pulmonary Specialized Center of Research.

From the Cardiovascular Research Institute and the Division of Clinical Pharmacology, Department of Medicine, University of California, San Francisco.

EXTRACELLULAR MATRIX MICROFIBRILS ARE COMPOSED OF CORE PROTEINS COATED WITH FIBRONECTIN

Extracellular proteins of cultured calf aortic smooth muscle cells consist predominantly of microfibrils 10-20 nm in diameter typical of "elastin-associated" microfibrils described in many tissues. Chemical and immunochemical evidence is presented that microfibrils consist of at least two proteins: core protein and fibronectin. Insoluble proteins of the microfibrils were obtained in the form of a pellet and antibodies raised in rabbits against these components. The antisera reacted with the insoluble microfibrillar proteins and with soluble fibronectin in enzyme-linked immunosorbent assay, and immunostained the extracellular microfibrils in cultured cells. An immunoglobulin (Ig) fraction was prepared and absorbed with fibronectin. The absorbed IgG retained its reactivity with the microfibrillar proteins but was no longer reactive with soluble fibronectin. Immunofluorescence studies were carried out using the absorbed IgG and IgG to soluble fibronectin. Both antibodies showed immunoreactive microfibrils in the extracellular matrix of cells in log phage. However, with increasing time in culture, as the cells reached confluence, the immunofluorescence of microfibrils reacting with the absorbed IgG became less intense, whereas that of microfibrils reacting with IgG to fibronectin increased; in confluent cells, essentially no staining was detected with the absorbed IgG, and a dense network of intensely stained microfibrils was seen with IgG to fibronectin. Treatment of these cultures with urea led to partial dissociation of the fibronectin and increased visualization of the microfibrils with the absorbed IgG; double-label immunofluorescence showed that both proteins occurred on the same microfibrils. The localization of immunoreactive sites to the extracellular microfibrils was confirmed by immunoelectron microscopy. Nearly quantitative cleavage with CNBr failed to dissociate the antigenically active fragments of fibronectin from the CNBr fragments of the core proteins of the microfibrils. It was concluded that microfibrils contain core proteins and fibronectin that are codistributed in insoluble, possibly cova-

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